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OF

# EXPERIMENTAL ZOOLOGY

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## A LINKAGE VARIATION IN DROSOPHILA

CALVIN B. BRIDGES

*From the Zoological Laboratory, Columbia University*

In the breeding work upon *Drosophila* done in this laboratory, it has been the practice to allow a female to lay eggs only for a period of about ten days. This is the average length of time from the mating of a female to the emergence of her offspring. But this first brood does not by any means exhaust the eggs of a female; if she is transferred to a fresh culture bottle she will lay as many eggs in this as in the first, and will continue to lay for forty or fifty days. Ordinarily, then, we obtain a sample of from 200 to 400 flies from a female, although three times as many might be obtained. It seemed to me that the full output of each female would give a truer index than the one that we were using. Accordingly, in working out the linkage relations of several mutations, I raised from each of the  $F_1$  females of a few experiments a second brood.

In cases involving the second chromosome a remarkable relation came to light when the results of the second broods were compared with those from the first. There had been a change in the linkage so that both in the totals for each experiment and in a great majority of the individual cultures the percentage of crossing-over had fallen significantly. Or, in other language, the 'coupling strength,' or 'gametic ratio' had risen. This change, while very interesting theoretically, promises further to become an aid in the study of the mechanism of linkage.

In the case of the first (sex) chromosome a large amount of data shows no change from first to second broods.

In the case of the third chromosome present data show an increase in the percentage of crossing-over, but because of the small number of cases the rise may not be significant.

The most efficient experiment by which to determine the amount of crossing-over between gens is the back-cross. Here a multiple heterozygote is tested by mating to the corresponding multiple recessive. When this is done, in the next generation produced, there are two contrary classes representing the original or  $P_1$  combinations, and two other contrary classes of crossovers. The percentage of crossing-over is given directly by division of the sum of the crossover classes by the sum of all the classes. It was in this way that the percentage of crossovers was calculated for the following tables.

TABLE I  
*P<sub>1</sub> purple vestigial × wild.      B. C. F<sub>1</sub> wild type ♀ × purple vestigial ♂♂*

REF.	NON-CROSSOVERS		CROSSOVERS		TOTAL	PER CENT OF CROSS-OVERS	$\Delta$
	Purple vestigial	Wild type	Purple	Vestigial			
A	178	202	16	16	412	7.8	
A'	152	227	13	14	406	6.6	-1.2
B	91	100	18	13	222	14.0	
B'	69	104	12	8	193	10.3	-3.7
C	165	150	17	19	351	10.3	
C'	191	216	18	17	442	7.9	-2.4
D	140	149	20	15	324	10.8	
D'	116	122	9	4	251	5.2	-5.6
E	191	214	20	19	444	9.0	
E'	196	229	11	22	458	7.2	-1.8
F	202	226	20	22	470	8.9	
F'	197	228	25	20	470	9.6	+0.7
G	105	158	17	17	297	11.4	
G'	188	232	17	14	451	6.9	-4.5
H	123	140	26	30	319	17.6	
H'	129	179	11	20	339	9.1	-8.5
1sts	1195	1339	154	151	2839	10.7	
2nds	1238	1539	116	119	3010	7.8	-2.9
Total.....	2433	2876	270	270	5849		

The mutant races used in these experiments will be fully described in a series of papers by Morgan and Bridges. In this paper I am considering only the small fraction of data in which we have records of more than one brood from a single female.

#### THE SECOND CHROMOSOME DATA

The first case in which this relation for the second chromosome was clearly shown was that of purple and vestigial given in table 1. Of the eight females whose tests are given in the table, seven showed a decrease in the percentage of crossing-over and one (F) showed an increase, which, however, was smaller in amount than any of the decreases. Likewise, the totals for the second broods when compared with the totals for the first broods showed a decrease of nearly three units in the amount of crossing-over. That differential viability has little or nothing to do with this difference is evident from the regular totals. This is shown even more clearly by the converse case ('repulsion'), where the difference is entirely negligible. In all of these experiments contrary classes are affected similarly and to a like degree, which would not be the case if viability were causing the change.

Of the six females tested (table 2) all showed a decided drop, and the totals show a rather greater drop (5 units) than in the 'coupling' case.

In obtaining data upon any linkage case it is best to have half the data in the form of 'coupling' and half as 'repulsion' experiments. It has been shown by comparative breeding tests that differential viability can be to a great extent eliminated by careful attention to the conditions of breeding—particularly by breeding in pairs in large culture bottles with the right amount of well prepared food. We may offset even this remaining disturbance by balancing the viability of a certain class against itself. For example, let us say that in the case of purple vestigial, the class vestigial is poorly viable. If, then, vestigial occurs in an experiment as a crossover class, that class will be too small and a false linkage value will be obtained. The remedy is to

balance against these flies an equal number in which vestigial occurs as a non-crossover. In this case the error will be the opposite of the previous one, and by combining the two experiments the errors should balance and give a better approach to the true value. If each class is thus balanced the error should

TABLE 2  
*P<sub>1</sub> purple × vestigial. B. C. F<sub>1</sub> wild type ♀ × purple vestigial ♂♂*

REF.	NON-CROSSOVERS		CROSSES		TOTAL	PER CENT OF CROSS-OVERS	Δ
	Purple	Vestigial	Purple vestigial	Wild type			
I	157	178	26	21	382	12.3	
I'	200	165	12	14	391	6.7	-5.6
J	198	176	23	23	420	11.0	
J'	242	195	19	26	482	9.3	-1.7
K	252	227	34	38	551	13.1	
K'	198	178	26	20	422	10.9	-2.2
M	205	158	27	32	422	14.0	
M'	213	246	14	23	496	7.4	-6.6
N	66	54	6	11	137	12.4	
N'	66	64	4	7	141	7.8	-4.6
O	189	172	30	32	423	14.6	
O'	217	225	13	18	473	6.5	-8.1
1sts	1067	965	146	157	2335	13.0	
2nds	1136	1073	88	108	2405	8.1	-4.9
Total.....	2203	2038	234	265	4740		

TABLE 3  
*Linkage of purple and vestigial with balanced viability (1sts)*

CLASS	NON-CROSS-OVERS	CROSSES	TOTAL	PER CENT OF CROSSES
Wilt type.....	1339	157		
Purple.....	1067	154		
Vestigial.....	965	151		
Purple vestigial.....	1195	146		
Total.....	4566	608	5174	11.8

be very small. If an equal amount of data for 'coupling' and 'repulsion' be combined, each possible class will appear in the required manner both as a non-crossover and as a crossover. Table 3 combines in this manner the results for the first broods of tables 1 and 2.

Of the 5174 flies of the first broods 608 or 11.8 per cent were crossovers. A similar balancing of the data of the second broods from tables 1 and 2 was made for comparison with the results of the first broods (table 4).

TABLE 4  
*Comparison of firsts and seconds (purple and vestigial) with balanced viability*

	NON-CROSS-OVERS	Crossovers	TOTAL	PER CENT OF CROSS-OVERS	$\Delta$	PER CENT $\Delta$
1sts	4,566	608	5,174	11.8		
2nds	4,984	431	5,415	8.0	-3.8	-32.
Total.....	9,550	1039	10,589			

If we take 11.8 units as the standard amount of crossing-over, the fall of 3.8 units is 32 per cent of this original amount of crossing-over. This fall of a third in amount, in connection with the fall in thirteen out of fourteen cases, shows that a real variation in linkage has occurred.

In this case the second broods gave a trifle over half of the 10,589 flies, showing that the egg-laying powers of the females had not diminished.

The case upon which there are the fullest data (16,873 flies in back-cross experiments) is that of black and curved. The first experiment involves 'coupling' (table 5).

Of the broods later than the firsts, four showed a decrease and two a smaller rise. In the totals the fall of 1.8 units is not large enough to be significant in itself. In culture 29 the female was carried through four broods and showed very little decrease in the number of offspring. Likewise the mother of 31 maintained her output. It is interesting that the fourth brood of 29 showed more crossing-over than did the first. Perhaps the fall reaches a maximum in the second broods. This question

TABLE 5

*P<sub>1</sub> black curved × wild.    B.C. F<sub>1</sub> wild type ♀ × black curved ♂♂*

REF.	NON-CROSSOVERS		Crossovers		TOTAL	PER CENT OF CROSS- OVERS	Δ
	Black curved	Wild type	Black	Curved			
29	102	103	34	40	279	26.5	
29'	72	107	27	24	230	22.2	-4.3
29''	103	142	36	42	323	24.1	-2.4
29'''	84	65	29	30	208	28.3	-1.8
30	105	106	27	44	282	25.2	
30'	127	149	44	32	352	21.6	-3.6
31	127	112	34	55	328	27.2	
31'	61	74	18	33	186	27.4	+0.2
31''	98	110	38	31	277	24.9	-2.3
1sts	334	321	95	139	889	26.3	
Others	545	647	192	192	1576	24.5	-1.8
Total.....	879	968	287	331	2465		

TABLE 6

*P<sub>1</sub> black × curved.    B.C. F<sub>1</sub> wild type ♀ × black curved ♂♂*

REF.	NON-CROSSOVERS		Crossovers		TOTAL	PER CENT OF CROSS- OVERS	Δ
	Black	Curved	Black curved	Wild type			
44	144	150	55	45	394	25.4	
44'	142	123	31	26	322	17.7	-7.7
48	134	119	53	41	347	27.1	
48'	159	125	32	35	351	19.1	-8.0
50	157	148	40	56	401	23.9	
50'	125	115	37	33	310	22.6	-1.3
52	135	95	29	47	306	24.8	
52'	105	100	21	42	268	23.5	-1.3
1sts	570	512	177	189	1448	25.3	
2nds	531	463	121	136	1251	20.5	-4.8
Total.....	1101	975	298	325	2699		

is to be investigated. In the 'repulsion' experiment (table 6) each of the four females showed a marked decrease, and the totals show a decrease of nearly five units.

The most convincing experiment is the following, in which three second chromosome loci (namely, black, purple, and curved) are run together in the same back-cross experiment. Such an experiment is much more satisfactory than three separate experiments would be in studying linear arrangement. In spite of the labor of getting the triple recessive used, and the time of classifying the flies in a more complex manner, there are many advantages. Since the same data furnish three linkage values, only a third as many individuals need ultimately be raised, or three times the amount of data may be obtained in the same time. The resulting values are more exactly and safely comparable, since they were produced under the same conditions. The order of gens is most strikingly shown by means of the smallness of the two contrary classes which are the result of double crossing-over. The amount of double crossing-over can be directly observed instead of being calculated from linkage values obtained in three separate experiments and perhaps not strictly comparable. A comparison of the observed amount of double crossing-over with the expected amount gives a measure of the amount of interference. Finally, if there is any disturbance of the linkage, it is important that the different values be derived from the same experiment, so that the disturbance can be shown to be localized or to affect the whole chromosome alike, as in the present case (table 7).

In the totals of the broods the change for each value is significantly a fall. The thirty-five cases of changed ratios are distributed as shown in table 8. In this table the percentage of decrease are calculated from the brood totals of table 7. It is evident that the fall has affected each section of the chromosome by approximately the same amount.

Of the twelve families of table 7, eight showed a fall and two a rise in both component values (black purple, and purple curved). It might therefore seem that there is some correlation, such that when black purple changes greatly purple curved

would change greatly also. Calculation showed that the change for black purple in any particular female differs on the average from the change for purple curved in that same female by 3.5 units. But the change for black purple in any given female

TABLE 7

*P<sub>1</sub> black purple curved × wild.    B. C. F<sub>1</sub> wild type ♀ × black purple curved ♂♂*

REF.	NON-CROSS-OVERS			SINGLE CROSSES			DOUBLES			PER CENT OF CROSSES BETWEEN						
	B	Pr	Cv	B	Pr	Cv	B	Pr	Cv	Total	Black and purple	Δ	Purple and curved	Δ	Black and curved	Δ
	Black purple curved	Wild type	Black Purple curved	Black purple curved	Black purple curved	Curved	Black curved	Pr	Cv							
58	63	62	4	9	17	15	1	4	—	175	10.3	—	21.1	—	25.7	—
58'	80	95	3	2	18	14	—	—	—	212	2.4	-7.9	15.1	-6.0	17.5	-8.2
60	131	163	12	5	41	34	4	3	393	6.1	—	20.9	—	23.4	—	—
62	148	150	12	5	33	39	1	1	389	4.9	—	19.1	—	22.9	—	—
62'	102	114	7	3	21	20	1	—	268	4.1	-0.8	15.7	-3.4	19.0	-3.9	—
66	147	156	9	2	31	26	1	5	377	4.5	—	16.7	—	18.0	—	—
66'	77	104	2	3	13	15	—	—	214	2.3	-2.2	13.1	-3.6	15.4	-2.6	—
68	89	76	6	7	24	24	3	—	229	7.0	—	22.3	—	26.6	—	—
68'	80	86	2	5	18	9	1	—	201	4.0	-3.0	13.9	-8.4	16.9	-9.7	—
70	92	92	7	1	17	26	1	—	236	3.8	—	18.7	—	21.6	—	—
70'	70	92	2	4	11	5	—	—	184	3.3	-0.5	8.7	-10.0	11.9	-9.7	—
72	129	153	7	10	34	29	—	4	366	5.7	—	18.3	—	21.8	—	—
72'	69	81	5	3	16	12	1	2	189	5.8	+0.1	11.4	-6.9	19.0	-2.8	—
74	103	121	9	4	25	23	1	1	287	5.2	—	17.4	—	21.2	—	—
74'	79	97	9	5	18	12	—	1	221	6.8	+1.6	14.1	-3.3	19.9	-1.3	—
76	122	102	12	9	28	20	1	2	296	8.1	—	17.2	—	23.3	—	—
76'	75	97	4	3	13	11	3	1	207	5.3	-2.8	13.8	-3.7	15.0	-8.3	—
78	140	143	6	7	26	33	2	1	358	4.5	—	17.3	—	20.1	—	—
78'	110	138	5	5	15	16	2	1	292	4.5	—	11.7	-5.6	14.1	-6.0	—
80	133	140	6	3	26	24	2	—	334	3.3	—	15.6	—	17.7	—	—
80'	81	89	7	3	19	15	—	1	215	5.1	+1.8	16.3	+0.7	20.2	+3.5	—
86	76	103	1	9	14	18	1	—	222	5.0	—	14.9	—	18.9	—	—
86'	96	83	2	1	10	14	—	1	207	1.9	-3.1	12.1	-2.8	13.0	-5.9	—
88	103	116	5	3	23	19	1	2	272	4.0	—	16.5	—	18.4	—	—
88'	109	111	7	4	28	23	1	—	233	4.2	+0.2	18.4	+1.9	21.9	+3.5	—
1sts	1476	1577	96	74	339	330	19	23	3934	5.4	—	18.1	—	21.3	—	—
2nds	1028	1187	55	41	200	166	9	7	2693	4.2	-1.2	14.2	-3.9	17.2	-4.1	—
Total...	2504	2764	151	115	539	496	28	30	6627							

TABLE 8  
*Cases from table 7 of change in linkage*

	BLACK	PURPLE	PURPLE CURVED	BLACK CURVED	TOTAL
Decrease.....	7		10	10	27
Increase.....	4		2	2	8
Percentage of decrease....	22.2		21.5	19.3	

differs from the change for purple curved in *any* female by 3.8 units, which is practically the same amount. From this point, that there is a negligible correlative change for the values for black purple and purple curved, we obtain confirmation of the view that this change is a general one which affects different sections of the chromosome independently, but on the average to about the same extent.

From the percentages of occurrence of the three mutants (table 9) it can be seen that differential viability has been almost entirely eliminated. In the case of black, 98 flies hatched for every 100 expected—a very close approach to expectation. In the second broods the viability is somewhat poorer, but since the decreases are uniform no changes in linkage are to be expected from that source.

TABLE 9  
*Viability coefficients (data of table 7)*

	BLACK	PURPLE	CURVED
1sts.....	0.98	0.97	0.97
2nds.....	0.95	0.95	0.94

The results in table 7 are not balanced here by an equal amount of data for each of the converse experiments. To balance the data of an experiment involving three loci requires four sets of data, instead of two, as in the case of two loci.

Fortunately, in the case of black and curved there are available data for such a balancing as is given for purple and vestigial in table 3. Only flies in first broods can be used for this balancing, as I shall make clear in the conclusion.

In a paper by Bridges and Sturtevant (Biol. Bull. '14), there appear records of 7419 such first brood flies in 'coupling' and

'repulsion' experiments. The data presented in this paper bring this total up to 11,353 flies and make the amounts for 'coupling' and 'repulsion' almost exactly equal, as shown in table 10.

TABLE 10  
*Linkage of black and curved (1sts) with balanced viability*

	NON-CROSS-OVERS	Crossovers	TOTAL	PER CENT OF Crossovers
Wild type.....	2210	644		
Black.....	2292	619		
Curved.....	2148	630		
Black curved.....	2147	663		
Total.....	8797	2556	11,353	22.5

The last case on which I can now present data for the second chromosome is that of streak and morula. Streak is a dominant mutation which occupies a chromosome position far from black in the opposite direction (left) from the loci occupied by the other mutants so far treated. Morula occupies a locus likewise very far from black but in the opposite direction (to the right) so that a great section of the chromosome extending beyond the black curved section in both directions is tested by this experiment. Here also the single case so far tested showed a fall (table 11).

TABLE 11  
*P<sub>1</sub> streak ♀ × morula ♂. B.C. F<sub>1</sub> streak ♀ × morula ♂♂*

REF.	NON-CROSS-OVERS		Crossovers		TOTAL	PER CENT OF Crossovers	Δ
	Streak	Morula	Streak-morula	Wild type			
82	50	47	40	31	168	42.3	
82'	50	31	26	24	131	38.1	-4.2

In table 12, I have summarized by tables the cases for the second chromosome. Of the sixty tests, forty-nine showed a decrease from first to second broods. This decrease appeared uniformly in each experiment.

A more satisfactory method of summarizing the data is that of table 13, wherein the data have been collected according to the linked pair of gens tested. The relative number of

TABLE 12

*Cases of change of linkage in the second chromosome, from tables 1, 2, 5, 6, 7 and 11*

	TABLES						TOTAL
	1	2	5	6	7	11	
Decrease.....	7	6	4	4	27	1	49
Increase.....	1	0	2	0	8	0	11
Total.....	8	6	6	4	35	1	60

TABLE 13

*Change for each linked pair of gens*

	PURPLE VESTIGIAL	BLACK CURVED	BLACK PURPLE	PURPLE CURVED	STREAK MORULA	TOTAL
Decrease.....	13	18	7	10	1	49
Increase.....	1	4	4	2	0	11
Percentage of decrease	32.3	12.7	22.2	21.5	10.1	

cases of decrease and of increase for each pair is shown (table 13). The percentages of crossing-over calculated from the totals of the first broods and of the second broods for each pair of gens, have shown in every case a fall which is considerable in amount. Table 13 (last line) gives this decrease in amount calculated as a percentage fall from the value given by the first broods as a standard, as was done in tables 4 and 8. All of these methods show the same real change in the amount of crossing-over between second chromosome gens in the second brood as compared with the first brood from the same female.

#### THE SEX CHROMOSOME DATA

Although in the case of the first, or sex chromosome, the data are not as great in amount as for the second, the conclusion is quite certain that here there is no change in the amount of crossing-over with second broods (table 14).

Between vermilion and fused ('fused' is at the extreme right end of the known plotted chromosome) there is a large amount of crossing-over, and the totals show that the value for the second broods is practically identical with that of the firsts. The five females tested showed a fall in three cases, balanced by

a rise in the other two. More data on the same case is furnished by a triple experiment, which involves as the other locus, bar, a dominant mutant described by Tice (Biol. Bull. '14).

These data (table 15) add four cases of rise to the three given by table 14, and a corresponding four cases of fall to the two from the same source. The value for bar fused shows three cases of fall and five of rise. For the pair vermilion bar the cases stand four against four. The totals in every case show practically no change (table 16).

The section from cherry to forked includes nearly all of the known sex-chromosome, and for this whole distance there is no change in the totals, and the females are balanced two against two (table 17). In this last case, that of cherry and sable, the slight change is a fall in the amount of crossing-over.

TABLE 14

*P<sub>1</sub> wild ♀ ♀ × vermilion fused ♂ ♂. F<sub>1</sub> wild type ♀ × F<sub>1</sub> wild type ♂ ♂*

REF.	FEMALES		MALES			TOTAL ♂♂	PER CENT OF CROSS- OVERS	Δ
			Non-crossovers		Crossovers			
	Wild type	Wild type	Vermilion	fused	Vermilion			
52	96	30	25	16	11	82	32.9	
52'	176	64	59	24	19	166	25.9	-7.0
53	60	22	20	9	6	57	26.3	
53'	76	27	21	11	10	69	30.5	+4.2
54	88	38	35	14	16	103	29.1	
54'	60	20	22	8	9	59	28.8	-0.3
57	61	20	22	7	11	60	30.0	
57'	170	54	47	24	19	144	29.8	-0.2
58	128	55	37	14	10	116	20.7	
58'	144	64	38	16	15	133	23.3	+2.6
1sts	433	165	139	60	54	418	27.3	
2nds	626	229	187	83	72	571	27.2	-0.1
Total.....	1059	394	326	143	126	989	27.2	

The summary for the data on the sex chromosome is given in table 18, similar to table 13 for the second chromosome. The total of females tested shows 17 cases of decrease and exactly the same number of increase, so that we may safely conclude that there is no change here from first to second broods. The

TABLE 15

*P<sub>1</sub>* bar ♀ ♀ × vermilion-fused ♂ ♂.    B. C. *F<sub>1</sub>* bar ♀ × vermilion-fused ♂ ♂.

TABLE 16  
*P<sub>1</sub> cherry ♀ ♀ × forked ♂ ♂.*   *F<sub>1</sub> wild type ♀ × F<sub>1</sub> cherry ♂ ♂.*

REF.	FEMALES		MALES				TOTAL ♂♂	PER CENT OF CROSS- OVERS	Δ
	Cherry	Wild type	Non-crossovers	Cherry	Forked	Cherry forked	Wild type		
25	129	145	73	70	65	68	276	48.2	
25'	167	148	74	82	66	88	310	49.7	+1.5
36	96	88	52	52	35	51	190	45.2	
36'	57	76	41	32	24	30	127	42.5	-2.7
84	76	86	40	34	38	26	138	46.3	
84'	62	71	24	39	25	28	116	45.7	-0.6
85	114	86	43	78	41	53	215	43.7	
85'	98	95	48	63	52	46	209	46.8	+3.1
1sts	415	405	208	234	179	198	819	46.0	
2nds	384	390	187	216	167	192	762	45.8	-0.2
Total.....	799	795	395	450	346	390	1581	45.9	

TABLE 17  
*P<sub>1</sub> cherry ♀ ♀ × sable ♂ ♂.*   *F<sub>1</sub> wild type ♀ × F<sub>1</sub> cherry ♂ ♂.*

REF.	FEMALES		MALES				TOTAL ♂♂	PER CENT OF CROSS- OVERS	Δ
	Cherry	Wild type	Non-crossovers	Cherry	Sable	Crossovers	Cherry sable	Wild type	
55	131	101	63	52	38	48	201	42.7	
55'	94	96	52	31	29	30	142	41.6	-1.1
225	197	115	83	67	78	—	343	42.3	

TABLE 18  
*Change for each linked pair of genes (1st chromosome)*

	CHERRY SABLE	CHERRY WOOLY	VERMILION BAR	VERMILION FUSED	BAR FUSED	TOTAL
Decrease.....	1	2	4	7	3	17
Increase.....	0	2	4	6	5	17
Total.....	1	4	8	13	8	34
Percentage change....	-2.6	-0.4	+0.4	+0.8	+13.6	

sing'e exceptional rise in the percentage in the case of bar fused is probably not significant, since the same females gave no rise but a fall in the case of other gens, and the distance involved is so small (2.3 units) that a very few flies make a great apparent difference.

### THE THIRD CHROMOSOME DATA

For the third chromosome I am now able to report only the single case of pink and kidney (table 19). Here four of the five second broods showed rises, and only one a fall. The totals show a rise of 2.4 units, or of 17.1 per cent, on the basis of the first broods. This case in itself is too small for any conclusion to be drawn. It is possible, however, that in the case of the third chromosome a rise from first to second broods may occur.

TABLE 19

*P<sub>1</sub> wild × pink kidney.    B.C. F<sub>1</sub> wild type ♀ × pink kidney ♂♂*

REF.	NON-CROSSOVERS		CROSSOVERS		TOTAL	PER CENT OF CROSS- OVERS	Δ
	Wild type	Pink kidney	Pink	Kidney			
17	109	97	11	14	231	10.8	
17'	98	84	27	12	221	17.6	+6.8
21	131	104	18	18	271	13.3	
21'	123	117	30	12	282	14.9	+1.6
23	111	91	23	6	231	12.6	
23'	79	60	21	4	164	15.2	+2.6
25	85	92	24	17	218	18.8	
25'	107	90	14	22	233	15.4	-3.4
27	172	138	33	20	363	14.6	
27'	121	105	30	21	277	18.4	+3.8
1sts	608	522	109	75	1314	14.0	
2nds	528	456	122	71	1177	16.4	+2.4
Total.....	1136	978	231	146	2491	15.3	

## CONCLUSIONS

Only data which have been obtained under like conditions can be used in dealing with any problem such as comparative linkage. If in the construction of a chromosome diagram the value used for A-B was that calculated from first brood data, and the value B-C was based on the total output of a female, the prediction of new values from the diagram would be inaccurate, unless the linkage remained unchanged throughout the life of the female. But if the diagram is constructed wholly from first brood values, predictions will be accurate. The practical point to be derived from this study is the breeding of only one brood from each female, especially in the second chromosome work. Any other condition as a standard could not be fulfilled with certainty for any large body of data.

Linkage has been explained by Morgan on a chromosome basis, in accordance with the cytological evidence. It is assumed that gens occupy fixed positions, linearly arranged within the chromosome. In diploid groups each such linear series is represented by two homologous chromosomes, *A* and *a*, every locus in the one (*A*) corresponding to the same locus in its homologue (*a*). Before maturation homologous chromosomes become paired, side by side, and the members of each pair become twisted about each other. At some of the points of contact the two strands twist in two, as it were; moreover, the end of *A* fuses to the other end of *a* as they lie opposed. Any gens that were in strand *A* but on different sides of a chiasma point will emerge in different strands because of the crossing-over, and hence will be segregated to different gametes. It is obvious that the closer together in the strand any two given gens lie, the less is the chance that in any given maturation a chiasma will occur between them, the chiasmata being distributed according to chance. The basis of linkage is that two gens lie in the same chromosome so close together that in less than half the maturing germ cells a crossing-over takes place between them.

There are two simple ways in which this scheme could be modified to give the change in linkage here described. We

must suppose that normally the tightness of twist for any chromosome varies, within limits, in the different maturing cells. The length of the section between nodes—the internode—will hence vary correspondingly, but around a modal length normal for that chromosome. It is also probable that crossing-over does not take place at every node but only in a certain percentage, specific for the chromosome.

If the average length of the section between nodes, the internode, remains unchanged (that is, if twisting becomes neither looser nor tighter) such a decrease in the amount of crossing-over between given gens can be explained as failure to break and re-fuse, i.e., cross over, at as many of the nodal points as normally.

If, on the other hand, the average length of the internode becomes greater (that is, if twisting becomes looser) such an effect as described would be produced while the percentage of breaks per node remains constant. A possible means of determining which of these views obtains here is offered by a study of the interference effects in the first and second broods.

Interference stands in about the same relation to linkage as linkage does to free Mendelian assortment. In linkage there is a hindrance to the independent assortment of two pairs (*A, a* and *B, b*) of allelomorphic gens. Such a case in sweet peas is that of round pollen versus long (pair *A, a*) and red flower versus purple (pair *B, b*). In the phenomenon of interference there is a hindrance to the independent linkage of the members of two couples of linked genes, *A-B* and *C-D*. In any case of free Mendelian assortment one expects as great a percentage of *A* to be at the same time *b*, as of *a* to be at the same time *B*; that is, *AB : Ab :: aB : ab* (as, e.g., in 9 : 3 : 3 : 1 or 1 : 1 : 1 : 1). In cases of linkage this relation is altered by a deficiency of the classes arising by crossing-over. Likewise in cases where two couples *A-B* and *C-D* in the same linkage group (chromosome) are crossed together, the number of individuals in the double crossover class may be considerably smaller than expectation according to the proportion above.

The development of the idea of interference is an illustration of the advantages of the chromosome hypothesis. The existence of this phenomenon was originally deduced by Muller and Sturtevant from a consideration of linkage as a chromosome process.

Linkage had been explained as the result of two loci in the same chromosome being so close together that a chiasma occurs between them infrequently. If the chiasma must occur at a node, then, since the internode has considerable length, there must be on either side of the chiasma space which is free from crossing-over. A couple of gens *C-D* might lie at such a distance from *A-B* that when a chiasma occurred between *A* and *B*, the length of the internode would cause the next chiasma to occur most often to one side of *C-D* rather than between *C* and *D*. In any one maturing egg a chiasma between the members of one such couple would tend to prevent one between the members of the other couple; consequently, few gametes would be formed which would be the result of both occurring simultaneously, that is, *double-crossing-over would be interfered with*. While the phenomenon of interference is thus a corollary of the chromosome hypothesis, it is almost unexplainable upon any other view of linkage.

Since the accuracy of the calculation of the amount of interference depends upon the accuracy of the smallest class—the double crossovers—in practice we derive the index of interference from a comparison of the observed percentage of double crossovers with the percentage which would be expected if there were no interference. The percentage of double crossovers expected without interference is the product of the total percentage of crossing-over between *A* and *B* by the like value for *C* and *D*. That is, if there is 5 per cent of crossing-over between *A-B*, and 10 per cent between *C-D*, then 5 per cent of 10 per cent would give the percentage of cases in which both occur. The case is exactly similar in treatment to the 9 : 3 : 3 : 1 ratio of two freely assorting pairs of gens, where 25 per cent of all cases are *a* and 25 per cent are *b*, and 25 per cent of 25 per cent gives the percentage occurrence of *ab*. In order to obtain

a convenient index, the converse of interference namely coincidence, is calculated as the percentage which the observed double crossover class is of the expected value.

If, as described, interference is a function of the chromosome twist, then from observing how the change in linkage here reported affects interference, we can deduce the method by which the linkage has been altered—whether by a decrease in the percentage of breaks per node or by a decrease in the number of nodes, that is, by a looser twist.

If the twist remains normal and the decrease is due to a decrease from the normal percentage of breaks per node, then each linkage value will be reduced proportionally. Whenever a crossover does occur it occurs in the same position in which it would normally have occurred, so that within any given section of chromosome as great a percentage of crossovers would be doubles as in the normal condition. The effect is to make the new condition a replica of the old, except that every crossover value is reduced in a common ratio. In this sort of change in the mechanism, the interference would remain *unaltered*, for in the ratio of the expected percentage of double crossovers to the observed percentage, both terms decrease proportionally, so that the value is unchanged.

If, however, the average looseness of the twist is increased, a totally different result will be produced. Here the nodes become actually further apart, so that whenever crossovers occur they are no longer in the same average position as formerly but are wider spaced. It then requires a longer section of the chromosome in order that double crossing-over be possible. This means that for any definite section the number of double crossovers becomes less, that is, *interference rises*.

On this second hypothesis, then, the closer spaced in relation to the length of the internode the members of the pairs *A-B* and *C-D* are, the higher is the interference. The new condition should show the same interference as would be shown under the old condition of a shorter internode by genes correspondingly more closely spaced. By considering the amount of apparent displacement (the decrease in the percentage of crossing-over)

we might calculate how much interference should rise if that change be due to an equivalent looser twist. Thus an *experimental* method is provided for the analysis of the mode of twisting and the distribution of chiasmas, not only under the changed condition, but also under the normal condition as compared to the changed condition. It is, however, no small task to secure data for such a study, and in any other material than *Drosophila* the problem would be wellnigh hopeless of solution. At present the unfavorable case of black purple curved furnishes only enough data to give a suggestion as to the mode followed.

The data for the calculation of interference in the case of black purple curved are given in table 7 and for the first broods may be summarized as follows:

B	Pr	Cv	B	Pr	Cv	B	Pr	Cv	B	Pr	Cv
	3,053			170			669			42	
Percentage	77.61			4.32			17.4			1.07	

Here the total amount of crossing-over between black and purple is  $4.32 + 1.07$  per cent, and between purple and curved is  $17.4 + 1.07$  per cent. The expected percentage of double crossing-over is therefore 5.39 per cent of 18.07 per cent, which is 0.97 per cent. The observed percentage (1.07) of double crossovers was somewhat *larger* than this. Although the difference is so small that it may be due to chance fluctuation, yet it will be instructive to consider its meaning on the assumption that it is not due to chance. The actual increase of 0.1 per cent is a relative increase of 11 per cent over the expected 0.97 per cent (percentage of coincidence 111). But interference which *increases* the percentage of doubles is a reversal of the ordinary type and the explanation of this 'negative' interference is as follows:

The preceding considerations have applied to the case in which the length of the average internode is such that when one node lies between *A* and *B*, the next node will most often lie *beyond C-D*. This relationship between the relative position of the gens and the length of the internode is such that a chiasma between one couple will tend to prevent one between the other

couple, so that *positive interference* will result. Let us now consider two arbitrary points,  $M$  and  $N$ , so chosen that the distance between them is equal to the length of the average internode. Whenever a node chances to occur near  $M$ , then the next node will most often occur near  $N$ . That is, the chances of a crossover at either point are very greatly increased by the occurrence of one at the other point. In this case, instead of getting less than the expected percentage of double crossovers, we would expect, on the internode hypothesis, to get *more* than the expected percentage. Interference for two such points may be termed 'negative.'

The second broods of the black purple curved cross give the following data:

B	Pr	Cv	B	Pr	Cv	B	Pr	Cv
	2.215		96		366		16	
Percentage	82.24		3.57		13.6		.594	

From this we find that the interference is zero (percentage of coincidence 100). There has been an 11 per cent rise in interference concomitant with the decrease in crossing-over. This would suggest that the decrease in linkage here studied has been due to an increase in the length of the average internode rather than to a decrease in the percentage of chiasmata per node. Unfortunately, the number of double crossovers obtained is not great enough to establish this change in interference as significant rather than due to chance fluctuation—i.e., the variation is within the limits of probable error.



# THE RELATIVE EFFICIENCY OF VARIOUS PARTS OF THE SPECTRUM FOR THE HELIOTROPIC REACTIONS OF ANIMALS AND PLANTS

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## I. INTRODUCTION

While the older authors had treated the motile reactions of animals to light as an indication of their love for or antipathy to this kind of energy, one of us in 1888 pointed out that we are dealing in these cases with phenomena of orientation comparable to the orientation of plants to light.<sup>1</sup> In order to indicate the identity of the mechanism in both cases he proposed the same term for both, namely, heliotropism (or phototropism). Loeb stated in his first full pamphlet (1889)<sup>2</sup> that (if one source of light be given) the animals orient themselves so that their plane of symmetry falls into the direction of the rays of light, "whereby the symmetrical points of the surface of the body are struck by the light at the same angle." In 1897 the same writer expressed the idea that the action of light which caused the heliotropic reactions was chemical.<sup>3</sup> Since it is reasonable to assume that symmetrical elements of the surface of the body are not only morphologically but also chemically alike, we must suppose that if the symmetrical elements of the surface of the animal are struck by the rays of light at the same angle, the velocity of the photochemical reactions in symmetrical elements of the surface (e.g., the eyes or skin) are the same, since the intensity of the illumination of a surface element varies with the cosine

<sup>1</sup> Loeb, J. *Sitzungsb. d. Würzburger physik.-med. Gesellsch.*, 1888.

<sup>2</sup> Der Heliotropismus der Tiere und seine Übereinstimmung mit dem Heliotropismus der Pflanzen. Würzburg, 1889.

<sup>3</sup> Loeb, J. *Zur Theorie der physiologischen Licht- und Schwerkraftwirkungen. Pflüger's Archiv*, Bd. 66, p. 439, 1897.

of the angle of incidence. The influence of the light upon the tension and action of symmetrical muscles must in such a case be identical. The light, if it remains constant, will therefore not cause the animal to alter the direction of its motions. If, however, the light strikes symmetrical elements at a different angle the velocity of photochemical reaction is not the same in the two symmetrical elements and the symmetrical muscles on both sides of the animal will receive unequal impulses (by reflex) from this source. This will lead to an automatic turning of the animal until its plane of symmetry again falls into the direction of the rays of light.

If these premises were true, it followed that the heliotropic reactions of animals should obey the law of Bunsen and Roscoe which says that (within certain limits) the photochemical effect of light is equal to the product of the intensity into the duration of illumination; and one of us predicted that this law would probably be found to hold for animal heliotropism.<sup>4</sup> This prediction proved true for the heliotropic curvatures of Eudendrium, as the experiments of Loeb and Ewald<sup>5</sup> showed. Ewald could also show that Talbot's law holds for the orientation of the eye of Daphnia by light,<sup>6</sup> and Talbot's law is an expression of the fact that the physiological effect of light is equal to the product of intensity and duration of illumination.

The same law holds for the heliotropic reactions of plants, as Blaauw and Fröschl had shown.<sup>7</sup> It also holds for the human eye.<sup>8</sup> In all these cases it should be remembered that the law of Bunsen and Roscoe is a threshold law, inasmuch as it holds only within certain limits.

With the reduction of both groups of heliotropic reactions, those of animals as well as of plants, to the same law, namely, that of Bunsen and Roscoe, it is idle to consider further the idea that animals are led to the light because they are "fond"

<sup>4</sup> Loeb, J. The mechanistic conception of life. Chicago, 1912.

<sup>5</sup> Zentralbl. f. Physiol., Bd. 27, p. 1165, 1914.

<sup>6</sup> Science, N. S., vol. 38, p. 236, 1913.

<sup>7</sup> Blaauw, Rec. des Travaux Botaniques Néerlandais, Bd. 5, p. 209, 1909; Fröschl, Sitzungsber. d. Akad. in Wien, 1908.

<sup>8</sup> Charpentier, Arch. d'Ophthalmol., tom. 10, 1890.

of it, or that they are turned away from it because they hate it; or that the reactions are the result of "trial and error."

We may, therefore, conclude that the heliotropic reactions of animals and plants are due to photochemical reactions and that the turning of the animal to (or from) the source of light is brought about automatically if the velocity of photochemical reaction is no longer the same in symmetrical areas of the photosensitive surface. This automatic turning results when the mass of photochemical reaction products on symmetrical points of the surface of the animal (eyes or skin) exceeds a certain value; and the variations of this value determine the relative sensitivity of different heliotropic animals. Since this has been stated more fully in former publications of Loeb we may refer the reader to these publications.<sup>9</sup>

If the basis of heliotropic reactions is a photochemical process, it follows that heliotropic animals must possess a photosensitive substance, and the question arises: Is this substance identical in all heliotropic organisms or do the photochemical substances differ in different heliotropic organisms? Especially does this question become of interest in respect to the question whether there is a specific difference between these substances in animals and plants.

The method to decide this question consists in comparing the relative heliotropic efficiency of different wave lengths in different organisms. If we find that the optimal heliotropic effects occur for one form of organisms in one kind of wave lengths, for another in a widely different wave length of the same spectrum, we may conclude that the photochemical substances in the two cases are different, if deduction be made for the possible screen effect of secondary substances contained in the sensitive organ.

The older experiments of the botanists were mostly made with colored screens, which yielded the result that behind red screens only weak or no heliotropic reactions of plants occur, while behind blue screens they occur as well as in mixed daylight. Loeb was able to show in his earlier experiments that the same holds

<sup>9</sup> The mechanistic conception of life. Chicago, 1912; article on Tropisms in Winterstein's *Handbuch der vergleichenden Physiologie*, Bd. 4, p. 451, 1912.

TABLE 1

DURATION OF ILLUMINATION, IN SECONDS	LOCATION OF THRESHOLD IN THE SPECTRUM, IN MICRA
6300	534
1200	510
120	499
15	491
5	487
4	478
3	—
4	466
6	448

good for the heliotropic reactions of animals. But these experiments are not adequate to decide the question of perfect identity of the photochemical substances in all cases. For this purpose experiments with spectral colors are required. The most reliable experiments made on plants are apparently those of Blaauw on the spore bearers of *Phycomyces* and the seedlings of *Avena*.

Blaauw proceeded in the following way: He exposed a row of seedlings of *Avena* to a carbon arc spectrum for a certain time. The seedlings were then placed in the dark and after the proper time it was ascertained which part of the spectrum had induced heliotropic curvatures. By varying the duration of time of exposure to the spectrum it was found that with a minimal time of exposure only certain blue rays, namely, those of a wave length of  $478 \mu\mu$ , caused heliotropic bending, while with longer exposure longer waves also became efficient. In this way the minimum duration of exposure for various parts of the spectrum was ascertained. Table 1 gives his result.

The red and yellow parts of the spectrum were ineffective for the intensity and time limits used and the optimum of efficiency was in the blue, in the region between  $466$  and  $478 \mu\mu$ .

A shorter series of experiments was made on the fruit bearers of *Phycomyces*, with the following results:

- 44 to 47 per cent of the *Phycomyces* showed heliotropic curvatures
  - after 192 seconds of illumination at  $615 \mu\mu$
  - after 192 seconds of illumination at  $550 \mu\mu$
  - after 16 seconds of illumination at  $495 \mu\mu$
  - after 32 seconds of illumination at  $450 \mu\mu$
  - after 64 seconds of illumination at  $420 \mu\mu$

The number of experiments was limited but they indicate an optimum between  $495$  and  $450\mu\mu$ , in this respect agreeing with the results on *Avena*.

We were anxious to know whether for the heliotropic reactions of sessile animals the optimum is situated in the same region of the spectrum. The number of sessile animals which are sensitive to light is rather limited and we had to make use of the hydroid *Eudendrium*, which also served in the experiments of Loeb and Ewald.

## II. THE HELIOTROPIC REACTIONS OF EUDENDRIUM

The newly formed polyps are positively heliotropic to light and they react by bending towards the light. The bending occurs in the region near the stem; the method of procedure was as follows:

Immediately after the colonies were brought into the laboratory good stems with from 4 to 8 or more polyps were selected. The polyps were cut off and the stems put into glass troughs filled with sea-water, where they were held in position by being fixed in little holes of a layer of paraffin, on the bottom of the trough. The troughs had plain parallel walls. The stems were exposed during the first day to ordinary light—since light is necessary for the regeneration of polyps<sup>10</sup>—and were then put into the dark-room. In the dark-room the polyps developed during the next day. These newly formed polyps are very sensitive to light and were used for the experiment. The trough was then exposed to a carbon arc spectrum, the visible portion of which was about 20 cm. wide. The spectrum was in a dark-room and all precautions were taken to guard against any reflected or other light from reaching the polyps. The stems were in a row and each one was exposed to a different part of the spectrum. The position of each individual polyp was marked in a diagram at the beginning of the experiment and the polyps were exposed to the spectrum for times varying from five minutes to five hours. Then the polyps were put into the dark again

<sup>10</sup> Loeb, J. Einfluss des Lichtes auf die Organbildung bei Tieren. *Pflüger's Archiv*, Bd. 63, p. 273, 1896.

and after the proper time (two hours or more) the number of the polyps which had bent to the light in various parts of the spectrum was ascertained. As the reader will notice, the bending of the polyps took place after they had been put back into the dark. This corresponds with the method followed by Blaauw in his experiments on plants and of Loeb and Ewald in their experiments on the applicability of the law of Bunsen and Roscoe to heliotropic reactions.

We have also made experiments in which the stems were exposed long enough to the spectrum to enable them to form their polyps while still exposed to the light. The determination of the wave length to which each stem was exposed was rendered possible through the use of the absorption bands of a solution of didymium nitrate. With the aid of these bands, we could define accurately the position of each stem and polyp in the spectrum. We are indebted to Dr. E. Butterfield for the exact location of these bands in the spectrum.

In noting the result the reader must keep the following facts in mind: When a short exposure to light influences the orientation of the polyps of a stem, this will show itself in the fact that the majority of the polyps of that stem will bend straight to the light. If there is no effect of light, the polyps will grow in any direction but it may happen according to the laws of probability that one or the other may bend to the light. In order to be sure that the light influences the direction in which the polyps bend we must require that so great a percentage bend toward the light that chance may be excluded, before we draw the conclusion that we are dealing with a heliotropic effect. We considered it a positive result when 50 per cent or more of the polyps bent to the light. That they should all bend to the light cannot well be expected, especially in cases of short duration of exposure. The new polyps are extremely delicate and they are not all healthy or strong. Moreover, certain polyps will be partly screened from the light by the stems. Blaauw, as well as Loeb and Ewald, had to use the bending of 50 per cent of the specimens as a criterion of a positive result. The fact that each stem has only a limited number of polyps creates

an additional difficulty, in this way: that if by chance one polyp is directed to the light and there are only five polyps on the stem it may appear as if 20 per cent of the polyps had reacted positively, while in reality the stem was not influenced by light at all. To avoid misinterpretations of this kind from influencing the interpretation of results we always give the number of polyps in a stem in the following tables.

In indicating the wave length it should always be remembered that the region given is usually the center of a small zone which contained the stem; since the latter is not straight and since the polyps are irregular in position it is not possible to indicate the position by one line in the spectrum.

We will now enumerate some experiments. In the first vertical column is given the wave length, in Ångström units, to which the stems were exposed (indicating in the next column the color of the region). In the third column we give the fraction of the number of polyps bending to the source of light over the total number of polyps. In the fourth column we give the percentage of the polyps bending to the light. Each experiment was made with different material on different days, unless the contrary is stated (table 2).

From this experiment we may deduce, first, that for this duration of exposure (five minutes) the rays to 5700 Å.u. (i.e., the

TABLE 2  
*Experiment 1: Exposure of *Eudendrium* polyps for five minutes to the spectrum*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
About 6500	orange-red	1/29	(4)
About 6000	yellow	0/4	0
About 5700	yellow	0/13	0
About 5300-5345	yellowish-green	5/15	33
About 5100	green	3/12	25
About 4900	blue	11/32	35
About 4735	blue	30/49	62
About 4690	blue	4/21	19
4600	blue	5/22	23
4400	indigo	5/52	10

orange and yellow rays) are absolutely ineffective; that the rays for 5300 to 4900 (i.e., yellowish-green, green, and greenish-blue) are only slightly effective; while the rays of the wave length of 4735 Å.u., (i.e., blue) constitute the *optimal* portion; and that in the indigo the efficiency diminishes again. This result is almost identical with the one obtained by Blaauw with the seedlings of oats (table 3).

TABLE 3  
*Experiment 2: Exposure, five minutes*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
About 5760	yellow	0/2	0
5711	green	1/2	(50)?
5100	bluish-green	4/6	66
4800	blue	9/22	41
4735	blue	3/3	100
4700	blue	3/11	27
4676	blue	8/10	80
4500	indigo	3/3	100
4400	indigo	2/2	100
	ultraviolet	0/11	0

This result was less striking than the previous one through the combination of two circumstances: (1) The material was more sensitive than that used in the previous experiment, and

TABLE 4  
*Experiment 3: Duration of exposure, five minutes*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
6200	orange	0/3	0
5600	yellow	0/5	0
5300	yellowish-green	1/10	(10)
5000	bluish-green	1/10	(10)
4850	blue	0/6	0
4735	blue	8/13	62
4700	blue	1/7	14
4450	indigo	0/3	0
4432	indigo	0/2	0
4000	violet	0/6	0
3850	violet	1/2	?
3600	ultraviolet	0/1	0

(2) the number of polyps was so small that the error was greater and the results not so uniform. Positive results were obtained in the region between Å.u. 4400 and 4735, that is, in the blue and indigo and also possibly in the bluish-green; yellow was ineffective, as before (table 4).

The most effective region of the spectrum in this experiment is again the region about 4735 in the blue, the same which has proved the most effective in the two previous experiments. In all the experiments red, orange and yellow, and extreme indigo and violet, were ineffective.

The next two experiments (4 and 5, tables 5 and 6) were made

TABLE 5  
*Experiment 4: Exposure to light, four minutes*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
About 5600	yellowish-green	0/5	0
5400	yellowish-green	6/23	23
5000	bluish-green	4/22	18
4800	blue	4/13	31
4735	blue	18/30	60
4700	blue	2/12	17
4670	blue	9/21	43

TABLE 6  
*Experiment 5: Exposure to light, three minutes*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
5760	yellow	3/11	27
5600	yellow	1/13	8
5200	green	2/16	13
4900	blue	2/4	50
4800	blue	4/10	40
4735	blue	3/3	100
4700	blue	7/20	35
4676	blue	0/1	0
4500	blue	0/1	0
4432	indigo	3/12	25
4100	violet	0/2	0
4000	violet	1/7	14
3800	violet	0/2	0

with shorter exposure of the polyps to the spectrum, namely, four and three minutes.

The exposure of three minutes is the minimum from which a result can be obtained, and the results of table 6 are difficult to account for. The time of exposure is so short that slight differences in the sensitiveness of various stems make themselves felt. Both experiments agree in their result with the previous ones, namely, that the region around 4735 Å.u. (in the blue) is the most efficient.

It was to be expected that in a longer exposure polyps would bend to the light in both indigo-blue and in green but not in yellow and red. Table 7 gives the result of an experiment with an exposure of fifteen minutes.

TABLE 7  
*Experiment 6: Exposure to light, fifteen minutes*

WAVE LENGTH IN ÅNGSTROM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
3700-4100	extreme violet	14/30	45
4100-4900	violet indigo and blue	72/95	76
4900-5400	green and bluish-green	14/37	38
5400-6700	orange-yellow toyellowish-green	0/?	0

Unfortunately, no record of the total number of polyps formed in the yellow and red was preserved; the number, however, was large.

The experiment confirms that the blue and indigo are the most efficient rays while the green are markedly less efficient. The yellow and red rays are inefficient. The longer exposure brings out the heliotropic effects in the extreme violet which do not show with shorter exposure.

In the following experiments an attempt was made to ascertain the influence of longer exposure. The first question was whether by making the duration of exposure considerably longer we should be able to induce heliotropic curvatures in the yellowish-green, yellow and red. Second, we wished to find out whether polarization effects might be observed in the case of too long an exposure.

The method was as follows: Different stems of *Eudendrium* with young polyps (prepared in the way described above) were put successively into the same limited part of the spectrum. Each stem was exposed a different length of time. The purpose was to find out how much time was required to cause the maximum number of polyps to bend toward the light (table 8).

TABLE 8  
*Experiment 7: Eudendrium exposed to light  
of 4700 Å.u. (blue)*

DURATION OF EXPOSURE IN MINUTES	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
5	4/25	16
10	8/11	73
20	8/29	28
40	1/3	33
80	18/23	78
160	24/24	100

The experiments show that an exposure of more than ten minutes (for the light intensity used in our experiments) did not essentially increase the percentage of polyps bending to the light.

TABLE 9  
*Eudendrium exposed to light five and a half hours*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
4800	blue	7/16	44
4950	blue	1/6	16
5300-5500	green	3/13	24
5720	yellow	0/21	0
6000-6550	orange and red	0/32	0

We made with the same material an experiment in which the polyps were exposed for five and a half hours to the spectrum from blue to red with the result shown in table 9.

The experiment shows again, first, that even in five and a half hours the rays from yellow to red are without any heliotropic effect. Second, that the efficiency of rays with wave length of

4800 and above is less than that of wave length 4700. It is possible but not certain that there are polarization effects.

In the next series of experiments a region in the neighborhood of 5600 Å.u. in the yellow towards the yellowish-green was selected for the stems (table 10).

TABLE 10  
*Experiment 8: Eudendrium exposed various lengths of time to the same wave length (about 5600 Å.u.).*

DURATION OF EXPOSURE IN MINUTES	POLYPS BENT TO THE LIGHT
10	0
20	0
40	0
80	0
160	0

In order to make sure that this negative result was not the fault of the material experiments with the same lot of material were carried out in the blue-violet part of the spectrum (table 11).

TABLE 11  
*Eudendrium exposed for one hundred and fifty minutes*

WAVE LENGTH IN ÅNGSTRÖM UNITS	COLOR OF THE SPECTRAL REGION	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
4220-4500	violet and indigo	21/24	87
4500-4660	indigo-blue	9/11	82
4660-4710	blue	7/10	70
4710-4750	blue	15/15	100
4750-4850	blue	14/16	85
4850-5000	blue-green	7/8	87

This experiment again shows strikingly that the blue and violet part of the spectrum is the effective one. The region between 4700 and 4750 Å.u. is again the most efficient.

In a third experiment of this series the stems were exposed to a wave length of about 4900 Å.u. (blue towards the green) for various periods of time with the result shown in table 12.

TABLE 12

*Experiment 9: Eudendrium exposed to light of  
4820 Å.u. (blue)*

DURATION OF EXPOSURE IN MINUTES	FRACTION OF POLYPS BENT TO THE LIGHT	PERCENTAGE OF POLYPS BENT TO THE LIGHT
0	2/9	22
10	5/11	45
20	8/12	75
40	7/11	64
80	11/13	73
160	5/18	28

With the same material an experiment with long exposure (five and a half hours) in the region of shorter wave lengths, 3750 to 4700 Å.u. (namely, from the blue to extreme violet) was carried out. In all, 52 hydroids out of 66 (i.e., 79 per cent) were bent forward. There was not much difference in the various regions, probably due to the long exposure.

### III. CONCLUSION AND SUMMARY OF RESULTS

These experiments have shown that the most efficient region in the spectrum for the production of heliotropic curvatures in the hydroid *Eudendrium* is situated in the blue at  $\lambda \sim 4735$  Å.u. This region coincides approximately with the one found by Blaauw for the seedlings of oats namely  $\lambda \sim 4780$  Å.u.

The regions in the red, orange and yellow are practically without effect in both *Eudendrium* and *Avena*.

The heliotropism of the sessile animal *Eudendrium* and that of the sessile plant *Avena* are therefore identical even as regards the most efficient wave length.

We expect to discuss the effects of different wave lengths upon the heliotropic reactions of motile animals and plants in another paper.



## THE ORIENTATION OF AMPHIOXUS DURING LOCOMOTION<sup>1</sup>

LESLIE B. AREY

Observers have differed regarding the question as to which end of *Amphioxus* is in advance during swimming. Rice ('80, p. 8) seems to have been the first to record observations on this subject:

These movements were executed sometimes upon the back, sometimes upon the abdomen in the position of ordinary fishes, it seemed to make very little difference which side was uppermost, but I have never seen them move backwards or tail-end foremost. After circumnavigating the vessel once or twice gradually moving slower and slower, they would stop and sink down upon the sand at the bottom.

In a more general statement Steiner ('86, p. 497) came to the same conclusion as Rice, "sie stellen sich so auf, dass ihre Breitseite in die verticale Ebene fällt und rasch entfliehen sie (from the stimulus) mit grosser Geschwindigkeit, das Kopfende voran, indem der Körper schlängelnde Bewegungen macht, an denen der Kopf nachweisbar theilnimmt." Two years later ('88, p. 41) he expressed the same opinion in almost identical language.

Parker ('08, p. 441) took the opposite view:

The locomotion of *amphioxus* is a rapid, curiously irregular wriggle, often accompanied with somersault-like movements which make it impossible to be sure at any moment whether the animal is swimming backward or forward. The results of momentary stimulation, however, show very conclusively that *amphioxus* can swim both backward and forward, and that the direction of swimming at the beginning of any course is dependent upon the part of the animal's body that was stimulated. But how long *amphioxus* keeps to one form of movement I was unable to discover. The fact that it usually buries itself in the sand tail first leads me to believe that, though it can swim forward, as maintained by Rice and by Steiner, it usually swims backward.

<sup>1</sup> Contributions from the Bermuda Biological Station for Research. No. 36.

Parker and Haswell ('10) in their "Textbook of Zoology" refer to the more or less upright position which *Amphioxus* assumes after burrowing in sand and then add the following astonishing and somewhat ambiguous statement (vol. 2, p. 46): "It also swims in the vertical position, - - - - .". However this assertion may be interpreted, it certainly is contrary to fact as far as the West Indian *Amphioxus* is concerned, and from the description of other writers (see also the frontispiece in Willey's '94 book), the same criticism undoubtedly applies to the closely allied European species as well. Anyone may easily convince himself that the foregoing quotation either presents a gross error or is highly misleading (according to the alternate possibilities of interpretation), if he will observe for a short time the locomotor responses, regardless of the antero-posterior orientation, exhibited by *Amphioxus* under various natural or experimental conditions.

While working recently at the Bermuda Biological Station opportunity was afforded me for making observations on the swimming habits of the West Indian lancelet, *Branchiostoma caribbaeum* Sundevall, a species very similar to the common European *Amphioxus*. This animal is found in abundance in the coarse coral and shell sand of Flatts Inlet, which connects the water of Harrington Sound with the outside ocean.

Ordinary mechanical stimulation, as by a finely drawn glass rod, gave too active a response for observation and accordingly a milder stimulus was sought. This was found in a weak stream of sea-water forced from a rather large canula; if the jet was weak and was directed vertically through 10 cm. of air and 10 cm. of water, the entire body of the animal became subjected to a gentle stimulus formed by the wave front. When thus stimulated the locomotor response tends to be less energetic and the disadvantages of local or directional stimulation are obviated, while it has a further advantage over other mild stimuli such as jarring the containing-dish, inasmuch as individual animals may be singled out for experiment and watched from the beginning of their course.

About thirty individuals were placed in three glass jars containing a layer of 'amphioxus sand' and were stimulated by the method just described. All responses in which the orientation was doubtful and those responses which included wild dashes or excessive somersaulting were disregarded; of the responses recorded, some were observed during the whole course and others were judged chiefly by the orientation at the beginning and particularly at the slowed-down finish of the swimming reaction. A tabulation of observations obtained in this manner is as follows:

Total number of responses.....	50
Anterior end in advance.....	41
Posterior end in advance.....	9

In a considerable number of animals the first movement was backward, but the direction was quickly reversed bringing the anterior end in advance; this condition will be discussed later in connection with other experiments.

When, however, *Amphioxus* are free to move in unlimited space, the somersaulting and the quick reversals of direction make accurate observations as to what is occurring during the response very difficult. To obviate this difficulty a large porcelain pan was partly filled with water, giving depths which varied from 0.5 cm. at the edge to 1.25 cm. at the center. The movements of *Amphioxus* under these conditions were as follows: When a stimulus (a bristle or finely drawn glass rod) was applied to the anterior end, the animal responded with a backward spring; if locomotion was now continued, this direction was not maintained for more than a few centimeters, for a quick reversal occurred, exceedingly difficult to follow, but which seemed to include a doubling end for end succeeded by a partial rotation of the long axis of the animal about its middle point; as a result the *Amphioxus* swam away with its anterior end in advance and usually at an acute angle with the direction toward which it was heading before stimulation occurred. During subsequent swimming these reversals occasionally occurred and as a result the posterior end of the animal might be in advance

for a short time; in this event, however, another reversal soon restored the former orientation and the normal direction of continued movement was plainly with the anterior end in front. When the posterior end was stimulated the animal sprang forward and if it continued to swim, it proceeded head foremost, although subsequent reversals usually occurred from time to time.

One lancelet of this set was especially instructive; after stimulation and the usual energetic response, it would continue swimming at a rate of about 1 cm. per second for a considerable distance without reversal; in several instances it more than circumnavigated the dish, a distance of over a meter, yet the anterior end was always carried in advance. Several other animals showed the same behavior but in a less degree; in general, after several responses the reaction was as long but less vigorous than that of a rested animal, and hence was easier to observe.

A circular trough 30 cm. in diameter, 0.5 cm. wide at the bottom and 1.25 cm. wide at the top was constructed by placing a porcelain pan, bottom up, inside a slightly larger pan; the purpose of this arrangement was to give the animal free swimming room but to limit its locomotion to one direction. The results were in agreement with those described previously; it was impossible to make an *Amphioxus* swim backward for more than a few centimeters before somersaulting and forward locomotion occurred.

A final method, which gave more conclusive evidence concerning the orientation of the animal during normal locomotion in unlimited space, consisted in treating one end of the lancelet's body with an *intra vitam* stain, whereby through direct observation one could be certain of the animal's orientation even during the wild dashes that often occur. Objections may be raised to the artificial conditions of the experiments described above, which were devised for limiting the animal's movements; thus it is entirely possible (although I do not believe it to be actually the case) that *Amphioxus* has a more complicated swimming behavior in the open than when locomoting in close quarters where some of its movements, including perhaps back-

ward swimming, are omitted. The whole locomotor response is undoubtedly much more rapid during unimpeded progress, hence it might be argued that although the animal in slower and more deliberate swimming carried its anterior end persistently in advance, yet when moving rapidly, if once reversed to backward swimming (and I have shown above that this reversal actually occurs from time to time), the physiological inertia of the animal as it travelled at its highest speed would tend to keep it so oriented until the swimming movements grew less energetic and the animal returned to the deliberate swimming that is characteristic at the end of the course. The data given at the beginning of the paper, in which nine out of fifty animals were recorded as oriented with the posterior end in advance while swimming freely in large aquarium jars, would tend to strengthen this suspicion; on the contrary, the actual results obtained from a study of stained animals did not substantiate such a line of reasoning.

If an animal, with the exception of one end, is wrapped in a fold of wet absorbent cotton and laid on a glass plate, the exposed end can be immersed without difficulty in the stain; in this case a weak solution of neutral red made up in sea-water was used. The anterior end was the one stained in most cases, for the more open structure of the pharyngeal region offers a larger surface for the reception of stain. After the stain had caused coloration to a deep pink or light reddish shade the animals were allowed to recover over night.

When stimulated after such treatment, the stained extremity could be followed with comparative ease, and observations made in this way corroborated my earlier conclusions. *Amphioxus* does not locomote backward for any considerable distance, even when the response is extremely vigorous; but, after a somersault brings it tail-end in advance, either another reversal follows directly or the animal changes its course and returns more or less in the direction from which it came.

I believe the observations recorded in the first experiment of nine lancelets, which were supposed to swim backward, can be explained as follows: When the swimming response is nearing

completion, the vigor of the muscular movements rapidly decreases and ends in complete collapse (Rice '80, p. 8; Parker '08, p. 441). After cessation of movement the animal is carried on a short distance by its own momentum and then sinks slowly to the bottom. According to notes taken at the time, five of the nine animals thus observed were judged chiefly by the finish of their response; if a reversal occurred just previous to the cessation of swimming, it is reasonable to expect that the nearly exhausted animal would not reverse again but would continue tail first with the last feeble strokes which precede complete exhaustion.

When *Amphioxus* has been kept in the laboratory for a short time the anterior half of many animals begins to turn pink and in a few days that end may become decidedly colored. This is presumably a manifestation of an approaching moribund condition, although the reactions of the lancelets appear to be practically normal. Observations of a number of *Amphioxus* in this condition led to conclusions similar to those gained by the study of artificially stained animals.

Referring to the quotations above, it will be seen that the views of Rice and of Steiner, although agreeing with mine in the main, show some differences. Rice's statement that he never saw an *Amphioxus* move 'tail-end foremost' is not only contrary to the results given in my tabulation, where nine out of fifty observed animals were so oriented during normal swimming, but is also not in accordance with Parker's ('08, p. 431; pp. 437-440) experiments, in which resting animals stimulated mechanically or chemically on the anterior end or mid-body, responded with a backward spring. Steiner's simple statement that *Amphioxus* locomotes 'das Kopfende voran' is certainly too general and omits entirely any mention of the characteristic backward movements just referred to in the criticism against Rice. My own observations agree perfectly with Parker's, to the effect that *Amphioxus* burrows in the sand tail first, but his belief, obtained as an inference from this habit, that the animal usually swims backward is directly opposed to the conclusion reached by me.

It is interesting, however, to see how near Parker was to the real solution of the matter, although it must be said that the whole question was not of major importance in his work; thus ('08, p. 431) he says:

When the anterior end of an amphioxus resting in a shallow dish of sea water was touched even lightly with the bristle, the animal usually sprang backward, though occasionally forward. The backward spring was often accompanied by a somersault-like movement, whereby the animal became turned end for end. When the stimulus was applied to the posterior part of the body, the result was almost invariably a forward leap.

The somersaulting is only mentioned by him in connection with the backward spring, and this I have shown is characteristic-ally present at the time of reversal to normal swimming, while in a forward leap it is unnecessary and is usually omitted.

The animals mentioned above, which swam slowly for a con-siderable distance in a pan of shallow water, afforded an opportunity to observe the movements of the body during locomotion. The head and tail were bent simultaneously toward the same side; the posterior of all the flexures, which is by far the most prominent, occurs approximately at the level of the atriorepore; the next prominent flexure is at about the region of the first gonadic pouches but is much less extensive than the former. When swimming slowly no other flexures are evident except a suggestion of one rather close behind the anterior flexure last described. The occurrence of the two largest flexures just anterior and posterior to the gonadic pouches suggests that these pouches materially increase the rigidity of the body throughout the region where they occur and thus actually determine the position of the major flexures. As might be expected, when a forward spring occurs the first flexure is initiated at the anterior end and muscular activity extends posteriorly like a wave; when an animal leaps backward the reverse is true.

As regards orientation during locomotion, I thus conclude that while *Amphioxus* can swim backward for short distances, its normal orientation in continued swimming is with the anterior end in advance.

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## STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL

### X. FURTHER DATA ON SOMATIC AND GENETIC STERILITY<sup>1</sup>

MAYNIE R. CURTIS AND RAYMOND PEARL

Some time ago one of the authors (Pearl '12) called attention to the fact that any single record of non-production or low production could not be accepted as evidence for the absence of the genetic factor for high production, since the failure of a bird to lay might be due entirely to somatic (physiological) causes. Later a detailed description of two such cases was published (Pearl and Curtis '14). In both of these instances the ovarian eggs were formed, but did not enter the oviduct. In one case the funnel mouth was too small to admit a full-sized mature yolk, and in the other there was an apparent lack of tone in the muscles of the oviduct and its ligaments. In both cases the yolks were ovulated into the body cavity and resorbed without causing any apparent disturbance in metabolism.

The purpose of the present paper is to record some recent observations on other cases of the same general nature.

#### MATERIAL

On September 1, 1914, there were in the Maine Station's flock of first-year birds, eight apparently healthy birds which had laid few or no eggs, and one which had laid well up to the beginning of the breeding season and then stopped laying. All of these birds were hatched between April 7 and May 21, 1913. Several were from high laying strains. In order to determine if possible the cause of the partial or complete sterility, these nine birds were killed and carefully examined. The observations

<sup>1</sup> Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 73.

on them confirm and extend our previous conclusions in regard to somatic and genetic sterility, and also in respect to the ability of a bird to absorb rapidly through the peritoneum yolks discharged into the body cavity.

#### DATA

Data on the nine cases of partial or complete sterility are given in table 1. From the figures in this table it is possible to compare the performance record of each individual, both with the anatomical condition of the sex organs at autopsy, and with her genetic expectation, judged by the performance record of her sisters.

#### SOMATIC STERILITY

Birds Nos. 141, 81, 364, and 383 belong to high producing families. Not one of them had a sister which laid less than thirty eggs before March 1 (Pearl '12). These birds themselves would, therefore, be expected to be good layers. Examination of the final columns of the table shows that Nos. 141, 81, and 364 could not lay for anatomical reasons.

The oviduct of No. 141 had burst near the upper end of the isthmus. In the body cavity was a yellow liquid composed evidently of egg mixed with serum. In this liquid were many short tubular pieces of egg membrane of approximately the length of the portion of the isthmus anterior to the tear. The opening in the wall was of such size that it was impossible for an egg to pass it. The oviduct on both sides of the tear was in normal laying condition. There was a normal egg (a yolk enclosed in thick albumen) in the posterior end of the albumen secreting region. In the ovary was a series of five normal yolks, the largest apparently mature, and seven discharged follicles. At the posterior end of the body cavity was an empty collapsed egg membrane on which was a thin layer of shell. The peritoneum was slightly thickened, so that it was translucent rather than transparent. The viscera were normal. The bird was evidently in perfect health.

TABLE I

## Showing data on nine cases of partial or complete sterility

CHARACTER	BIRD NUMBER						
	141	81	364	383	316	458	349
No. of eggs laid...	8	7	3	2	61	5	3
No. of nesting records.....	104	70	1	7	78	66	0
Notes on production	Eggs: Oct. 10, 12, 18, 19, 26, Dec. 18, Feb. 4, and 15	Eggs: Oct. 29, Nov. 20, 21, 24, 29, May 6, July 4	Eggs: Jan. 7, Feb. 8, June 20, Nesting record Apr. 22	Eggs: * Apr. 13, 15, 17, and 26, May 8, 13, 15, 17, and 26, All nesting records in Apr. and May mostly after Dec. 22 Mar. 1	Eggs: Feb. 2, Mar. 1-3, 13, 15, 17, and 26, May 26, All nesting records after Dec. 22 Mar. 1	Eggs: Feb. 24, 26, Mar. 1, 16, 19, 21, 25, 26, All nesting records in Jan., Feb. and Mar.	Eggs: Feb. 2, Mar. 1-3, 13, 15, 17, and 26, May 26, All nesting records after Dec. 22 Mar. 1
No. of sisters in laying house...	10	6	3	3	2	4	24†
No. of sisters with winter production of 30 or more eggs.....	10	6	3	3	1	2	10
No. of sisters with winter production of less than 30 and more than 0 eggs....	0	0	0	0	1	2	13
							25
							13

\*Each of these two birds had a normal egg in the oviduct and in neither case was there any apparent obstruction to the laying of the egg. It is, therefore, almost certain that these eggs would have been laid in a few hours. Further, the absolutely normal appearance of the peritoneal surface, and the absence of yolk or eggs in the body cavity, made it seem improbable that yolks had been recently ovulated which failed to become incorporated into normal laid eggs. However, bird No. 349 had one, and bird No. 383 three, discharged follicles which could not be accounted for from the egg in the duct and those laid in trap-nests within a period compatible with the state of resorption of the follicles. It seems probable then that these eggs were laid outside the trap-nests. In this connection it should be said that the nine birds here discussed were a part of a flock of forty-five selected from the laying-house during the last week in August and moved into a pen in the breeding-house. From August 29 to September 2 this flock laid sixty eggs in trap-nests and fourteen on the floor. The high proportion of floor eggs was no doubt due to two causes: (1) The birds had been moved into strange quarters, and (2) they were somewhat neglected during the sorting of the flock and cleaning of the houses.

† These birds had no full sisters in the laying-house. The figures in the table are for half-sisters by the same father.

TABLE I—*Continued*

CHARACTER	141	81	364	383	316	458	349	210	431	BIRD NUMBER
										4
Mean winter production of sisters.....	70.1	57.8	49.0	65.0	44.5	35.8	28.2	32.5	40.4	
Maximum sister's winter production.....	103	93	53	79	68	67	75	92	99	
Minimum sister's winter production.....	43	41	45	57	21	17	0	0	0	
Body weight in grams.....	2290	2891	2412	2511	3039	2070	2086	1682	1786	
No. of yolks above 1 cm.....	5	5	5	5	6	4	5	2	0	
Weight of yolk in ovary.....	40.5	55.0	42.5	44.0	58.5	35.0	41.5	5.0	0	
Weight of ovary without yolk.....	7.0	4.0	4.5	4.0	7.0	3.0	4.5	3.5	1.0	
No. of discharged follicles.....	7	3	5	4	3	7	4	0	0	
Length of oviduct in cms.....	69	68	80	75	78	56	74	75	22	
Weight of oviduct in grams.....	41.5	38.0	52.5	47.5	41.0	32.0	46.0	21.0	2.0	

TABLE I—Continued

CHARACTER	BIRD NUMBER					
	141	81	364	383	316	458
Cyclic condition of sex organs	Laying; egg in al-	Laying	Laying; egg shell gland	Laying	Laying	Laying; egg on isthmus
	bum region					turing
Obstruction to Oviduct torn	Lips of funnel	Cystic tumor	None found	Cystic tumor (wt. 23 gms.)	Ovary walled off	Intermediate between laying and non-laying
egg laying	used	oviduct				
Condition of yolk etc., in body cavity	Much yolk mixed with serum bathing viscera.	In small lumps	None found . . . . .	None found	In small lumps, A few very small lumps among viscera	On-laying
	Many short tubes of egg membrane in this liquid; a full sized empty collapsed membrane in posterior end of body cavity					
Condition of peritoneum	Slightly thickened	Slightly thickened	Normal	Normal	slightly thickened	Normal
					ended	

An examination of the bird's egg record shows that she started her year with two 2-egg clutches, and a separate egg in October, a record not unlike that of many birds which are good layers. There were only three eggs recorded after October 26; these were scattered; their dates of laying were December 18, February 4, and February 15. However, in November the bird began to go on the nest daily and the nesting records follow rhythms very similar to the rhythm of laying of a good egg-producer. The three irregular eggs recorded after October were in rhythms of nesting and may have been (and probably were) errors due to an egg having been left in the nest the last time it was used, or to the attendant accidentally recording an egg instead of a nesting record. An accurate determination of the time, or of the cause, of the rupture of the oviduct is impossible. It seems clear, however, that at the time it occurred there must have been a completed egg in the lower end of the duct. This egg was evidently carried up the duct and through the rupture into the body cavity by an antiperistaltic movement of the duct, which accompanied or almost immediately followed the rupture. This egg was evidently resorbed through the abdominal peritoneum, leaving the collapsed membrane found at autopsy. The nesting rhythm of the bird: the egg and serum mixture containing the short tubes of egg membrane found in the body cavity; and the normal naked egg in the lower part of the albumen-secreting region; all make it seem certain that after the rupture of the duct the sex organs passed through their normal cycles and that the eggs were formed in a normal manner, so far as the rupture of the duct allowed. After passing through the rupture they were absorbed directly through the general peritoneal surface.

Bird No. 81 had the two lips of the funnel tightly fused. In order to test this observation the duct was filled with water and the union of the edges of the funnel lips proved watertight except at one point at the lower angle of the mouth of the oviduct; through this the water slowly oozed; it was impossible for a yolk to enter the duct. Both ovary and oviduct were in laying condition. There were small lumps of absorbing

yolks among the viscera. In the ovary was a normal series of five growing yolks, the largest one mature; there were also three discharged follicles. It was apparent that the bird was ovulating into the body cavity and resorbing the yolk directly.

An examination of the egg record of this bird shows nesting records in a rhythm similar to a laying rhythm, and records of seven scattered eggs. These eggs occur in the nesting rhythms and, as in the case of the scattered eggs of No. 141, probably represent errors on the part of the attendant.<sup>2</sup>

The peritoneum was slightly thickened, as in No. 141. The fusion of the funnel lips may have been secondary, and due to peritonitis caused by accidental ovulation into the body cavity. However, there were no other visceral adhesions and no present evidence of sufficient peritonitis to cause adhesions. While it is impossible to say how long the oviduct had been closed, at the time of autopsy, at least, there was no possibility for a yolk to enter.

Bird No. 364 also had the sex organs in laying condition at autopsy. In the ovary was a series of five yolks and five follicles. Ten centimeters from the mouth of the funnel attached to the inner wall of the duct was a cystic tumor the size of a large egg yolk. There was no evidence of yolk or egg material in the body cavity. The visceral peritoneum, however, was thickened as in the birds which had been absorbing yolks. Evidently the bird had completely absorbed the yolk (or eggs) derived from the ruptured follicles. She had records of three scattered eggs, but only one nesting record. These recorded eggs may have been mistakes, but, since the hen was not nesting regularly,

<sup>2</sup> The difficulties of getting an attendant to look after trap-nesting operations on a large scale, who will consistently maintain the maximum level of possible accuracy (Pearl '11) are extremely great. During the past year we have been particularly unfortunate in this respect. While making every effort to do his best, the person who operated the trap-nests was psychologically poorly adapted to such work, and consequently the records are marred by a number of such easily detected errors as those referred to above in the cases of birds Nos. 81 and 141, and probably by some others not so readily detected. While this is a very regrettable circumstance, it really is not so serious as it might appear at first sight to be, since, after all, the total number of errors in the records is absolutely and relatively small.

the probability of getting credited with eggs she did not lay was much smaller than in the preceding cases. Since the maximum diameter of the duct is much greater than a normal yolk or the tumor it is possible that a few small yolks passed the tumor and became the yolks of normal laid eggs.

Bird No. 383 when killed was in normal laying condition, with an egg in the shell gland and a series of five normal yolks and four follicles in the ovary. There was no apparent obstruction to egg-laying. The peritoneum was normal and transparent. As explained in the footnote to table 1, it is probable that three eggs derived from the follicles on the ovary had been laid on the floor of the pen. The other was still in the duct. This bird's production record shows only three scattered eggs and seven nesting records. At the time of autopsy she was apparently in perfect health and capable of producing eggs. Why she had failed to lay was not apparent. The idea occurred to us that possibly she had been habitually laying on the floor. Investigation of the floor egg record for the pen in the laying house in which No. 383 had spent the year did not indicate that this was the probable explanation. The total number of eggs laid on the floor in this pen of 125 birds to March 1 was 92. This was not higher than the average number of unrecorded eggs for a pen of that size, and was not as high as many of the individual birds' trap-nest records. Also, the attendants pick up all birds seen laying on the floor and a habitual floor-layer is bound to be discovered in the long run. We must, therefore, attribute the failure of No. 383 to lay to some obscure physiological condition.

That there can be no reasonable doubt that No. 383 was genetically a high producer in constitution is shown by a careful examination of her complete record in this respect. Her sire was ♂ No. 623; all his daughters, which were hatched before June 1, with four exceptions to be noted below, made records before March 1, of over 30 eggs each:

Sire ♂ No. 623 ( $fL_1L_2$ + $fL_1L_2$ )		X	9 different ♀ ♀	
Winter production	Over 30	Under 30	Zero	
Observed	28	4	0	
Expected	32	0	0	
plus No. 383, the abnormal bird under discussion				

The four birds giving records under 30 laid respectively 26, 26, 23, and 19 eggs before March 1. These are undoubtedly to be regarded as somatic fluctuations from zygotes carrying the two factors<sup>3</sup> for high winter production (over 30 eggs). The bird which laid 19 eggs was not hatched till May 20.

Bird No. 316 belonged to a small family of which a part were high and a part low producers. By her own winter production of 55 eggs she had shown herself to be genetically a high producer. After the first of March she began to make many nesting and a few egg records; the last egg record was on April 21; the nesting records continued in normal laying rhythms.

Autopsy examination showed that there was a cystic tumor attached to the inner wall of the funnel mouth which practically closed the funnel; the oviduct was in laying condition; the ovary had a series of six large yolks and three follicles; there was some free yolk in the peritoneal cavity; the peritoneum was slightly thickened.

It was evident that this bird was normally a high producer, which had gradually developed a cystic tumor. This had at first hindered and finally prevented the entrance of yolks into the duct. From that time on the bird had gone through the normal laying cycles, ovulating the yolks into the body cavity and absorbing them.

Bird No. 458 belonged to a family which included both high and low producers. At autopsy the ovary was walled off from the rest of the viscera by a large peritoneal pocket which was attached to the dorsal body wall on all sides of the ovary. The pocket was bulging with the contained ovary and a considerable

<sup>3</sup>  $L_1$  and  $L_2$  of our former notation.

amount of free yolk. A little yolk was oozing through a small hole at the midventral angle of this pocket. There were a few small lumps of yolk among the viscera. When the pocket was opened and the free yolk wiped out the ovary was found to be in normal laying condition. It contained a series of four growing yolks, the largest mature, and seven resorbing follicles. The oviduct was in laying condition.

At the time of autopsy, then, this bird was ovulating into the ovarian pocket and absorbing the yolks. The rate of absorption was evidently not equal to the rate of yolk formation. In consequence the pocket was stretched to its capacity and had given way at one point, allowing a little yolk to escape into the body cavity.

The egg record of this bird shows neither eggs nor nesting records until December 22. From then on until the end of the year the nesting records follow a rhythm similar to a very slow laying rhythm. There is one egg recorded on February 2, a normal clutch of three on March 13, 15 and 17 and a single egg again on May 26. While it is possible that records of all these eggs are mistakes due to causes discussed in reference to the egg record of No. 141, it is probable that at least the record of the normal clutch in March is authentic, as the errors considered would hardly have resulted in this sort of a record. It seems probable that the pocket was neither congenital nor formed complete at once but that during its growth it first hindered and finally prevented the entrance of yolks into the oviduct.

In any case, the immediate cause of the partial sterility exhibited is somatic, in the sense that it is not directly connected with or related to the genetic constitution of the bird in respect to fecundity.

Bird No. 431 represents a case of somatic sterility of a different sort from those so far considered, in that the difficulty was not primarily with the genital organs. This bird had a crippled back which interfered with the normal use of her legs; she was in poor flesh; she had never been in a trap-nest either to lay or nest. At autopsy the sex organs were in strictly non-laying condition. There was no visible obstruction between the ovary

and oviduct. It is, however, improbable that the physiological tone of the bird had ever been sufficiently good to allow the formation of yolk. Genetically the bird might have been either a good or a poor layer.

#### GENETIC STERILITY

Bird No. 349 belongs to a family in which are individuals with, and individuals lacking, both factors for high fecundity. At autopsy this bird was in laying condition, with an egg in the oviduct and a series of five growing yolks and four discharged follicles in the ovary. Two of the three eggs recorded were laid respectively on the day of death and the second preceding day. These two eggs and the one in the duct account for three of the four follicles. The other may have furnished the yolk for a floor egg, explained as in the case of No. 383. There was no yolk in the body cavity; the peritoneum was normal. This bird had made no nesting records. Whether this bird was the extreme of genetically poor layers or whether her sterility was due to somatic causes too subtle for detection by rough autopsy examination is impossible to state absolutely. The probability, however, is extremely great that this bird genetically carries only  $L_1$  or  $L_2$ —that is, has only one dose of any of the factors on which production depends. This is evident from the following considerations:

	Sire ♂ No. 627 ( $fL_1L_2$ • $fl_1l_2$ )	×	9 different ♀ ♀
Winter production	Over 30	Under 30	Zero
Observed	10	13	1 (+No. 349, the bird under discussion)
Expected	9.6	12.8	3.2

Now the dam of No. 349 was No. 303 J, whose genetic constitution was  $fL_1L_2$ .  $fl_1l_2$ , with a winter record of 23 eggs, and a record for the year of 79 eggs. Absolutely the most likely result of mating such a bird with a Type 4 male, where, as in the present case, there is only one in the family, is a bird which will make a winter record under 30—that is, one which carries but one dose

of either  $L_1$  or  $L_2$ . The record of 1 egg for No. 349 on January 26 would, on a strictly literal interpretation, put her in the 'under 30' class. It seems clear, however, in view of the rest of her record, and of the fact, already repeatedly pointed out, that March 1 does not represent biologically the absolutely invariable time of beginning of the spring cycle of production, that she is really a zero winter producer. From a mating like that of ♂ No. 627  $\times$  ♀ No. 303 J one zero producer in every eight offspring is expected.

We may next consider the case of bird No. 249. This individual had no full sisters. The nature of the mating from which she was produced is shown by the following pedigree.

	Sire ♂ No. 628 ( $fL_1L_2$ + $fl_1l_2$ )	$\times$	11 different ♀ ♀
Winter production	Over 30	Under 30	Zero
Observed	21	25	4 (+No. 249, the bird under discussion)
Expected	19.15	24.98	5.83

In addition to the above, ♂ No. 628 had four other daughters, by three different females, which, because of the smallness of the families and for other reasons, cannot be exactly classified genetically. These four birds were all in the 'over 30' class.

We have classified No. 249 here as a zero winter producer because all of her 9 eggs were laid between February 24 and March 26. Further, all of the seven nesting records occurring during the early spring suggests that she is the extreme of the low producing segregates. It has been elsewhere pointed out that March 1 does not mark biologically a fixed limit of the winter cycle. Some birds, of which No. 249 is undoubtedly an example, begin their spring cycle a short time before that date.

Furthermore, the dam of No. 249, which was bird No. 436 J, was of constitution  $fl_1l_2.FL_1l_2$ , with a zero winter record, and a record for the year of 24 eggs. Such a bird, mated with a male like No. 628, should give one in every four daughters a zero producer.

The autopsy findings were in accord with this expectation. The sex organs were in a condition intermediate between laying and non-laying condition; no anatomical obstruction to egg laying was observed; there was no evidence that ovulation had taken place into the body cavity.

The case of No. 249 is to be regarded as one of nearly complete genetic sterility.

#### DISCUSSION

In the present discussion the term 'somatic sterility' is used to distinguish obstructions to egg-laying due to accidents or disease affecting the individual, and not (so far as we have any right to infer) inherited from her ancestors. A distinction is made between sterility due to these causes, which may include not only actual obstructive lesions of the genital organs, but also a general lowering of the physiological tonus of the individual to such an extent that it does not form yolk, and sterility due to a lack of the genes for egg-production.

The preceding paragraphs show that three of the four birds which belonged to high laying strains and which did not fulfill the expectation, based on a knowledge of their genetic constitution, failed because of the impossibility of a yolk entering the oviduct. Two other birds belonging to segregating families (one of these had proved herself a high producer by her own winter record) showed the same reason for not laying.

Another interesting observation is that four of the five birds known to be ovulating into the body cavity because of some obstructions to the mouth of the oviduct nested in rhythms comparable to the laying rhythm of normal birds. One of the authors (Pearl '12) has already published a similar record of another 'zero' bird belonging to a high laying line. There are now several similar records on file. He called attention to the fact that it has been experimentally shown at this laboratory that in cases of ligation, transsection or entire removal of the oviduct without injury to the ovary the

\* \* \* \* bird goes regularly through the entire process of laying save for extrusion of an egg which is physically impossible. The

'n' (nesting) record of such a bird is precisely like a normal egg record showing the same phenomena of rhythm and cycles. Each day's 'n' in the record of such a bird represents an egg which she would have laid, had she been physically capable of doing so.

We have later shown (Pearl and Curtis '14) that in all cases of surgical interference with the oviduct the ovary passes through the same rhythm as in unoperated birds. In such cases the formation of the egg proceeds as far as the obstruction to the oviduct.

The whole body of evidence is now so convincing that we cannot escape the conclusion that nesting records are, in the great majority of cases at least, associated with ovulation into the body cavity, or the backing into it of a partly or fully formed egg.

Patterson ('10) stated that "the stimulus which sets off the mechanism for ovulation is not received until the time of laying (in cases where birds are laying daily) or shortly thereafter." He bases this assertion on the fact that before the laying of the egg the oviduct is inactive, but "shortly after laying is in a state of high excitability with the infundibulum usually clasping an ovum in the follicle."

In view of the results set forth in No. VIII of these Studies, and in the present paper, it would appear probable that the connection, if there is any connection, between the instinct to nest and to lay (i.e., to expel the completed egg) and ovulation is the reverse of that implied by Patterson. Since birds which entirely lack an oviduct (Pearl and Curtis '14) and therefore cannot by any possibility lay an egg, still ovulate perfectly well and in a normal rhythm, egg-laying cannot very well be the stimulus to ovulation, as implied by Patterson. The explanation which accords best with our present knowledge is that the instinct to nest and to lay is the normal but not absolute (for example, note that No. 364 was ovulating into the body cavity and not nesting) *resultant* of ovulation, even in cases where the yolk does not enter the oviduct.

## SUMMARY

1. Birds which are hereditarily high layers may fail to make good performance records because for some anatomical reason it is impossible for yolks to enter the oviduct.
2. Birds which ovulate, or return partly-formed eggs, into the body cavity usually show the nesting instinct.
3. The nesting records show a rhythm similar to egg records of normal birds and it seems probable that they are the normal resultant of the ovulation.
4. Data given in this paper also confirm the following statements made in a recent paper (Pearl and Curtis '14):
  - a. In case of stoppage of the duct at any level, the duct on both sides of the point of stoppage passes through the same cyclic changes, coordinated with the cyclic changes in the ovary, as a normal unobstructed duct. The duct functions only as far as it receives the stimulus of the advancing egg.
  - b. Absence of pressure from the funnel does not prevent or apparently greatly retard ovulation. Increased internal pressure may therefore be the most important factor in normal ovulation.
  - c. Yolks of partly or fully formed eggs may be absorbed rapidly and in large numbers from the peritoneal surface without causing any serious derangement of normal metabolic processes.

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## BRISTLE INHERITANCE IN DROSOPHILA

### I. EXTRA BRISTLES

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### SIX FIGURES

The four bristles normally found on the dorsal surface of the thorax of *Drosophila ampelophila* form a rectangle. The extra bristles studied in the following experiments occur in the two longitudinal rows of the normal bristles, or just mediad or laterad to these rows. Figure 1, showing some random patterns of bristles, gives some idea of the different arrangements possible for the most common numbers of extra bristles. These patterns were drawn free hand, since the overlapping of the bristles prevented the use of a camera. The drawings are not exact, but the relative positions in the two rows are sufficiently accurate to indicate that it would be practically impossible to make more than a roughly approximate classification of flies according to patterns, even if a very large number of standard patterns were established. There appears to be no position in the two rows that may not be occupied by an extra bristle, thus making it impossible to develop any satisfactory homologies. This is especially true in the higher grades. For these reasons the best method seems to be recording merely the numbers of extra bristles. As will be seen, the results based on the data of bristle numbers shows very plainly that any attempt to obtain and use data of patterns of extra bristles would be futile with the present cultural methods.

All matings described below were made in pairs. The virginity of the females mated was determined by the pale pigmentation which deepens when the cuticula hardens a few hours after hatching. Accurate pedigrees and daily records of the sex and bristle number of each fly observed are on file. The results presented in this paper are based on 350 pedigree matings and bristle counts of over 54,000 flies.

Grateful acknowledgment must be made of the assistance rendered by Prof. T. H. Morgan in suggesting that the extra bristles found at times among his flies might form suitable material for the problem the author had in mind, and also in supplying numerous stocks of flies for observing the frequency of the occurrence of the extra bristles.

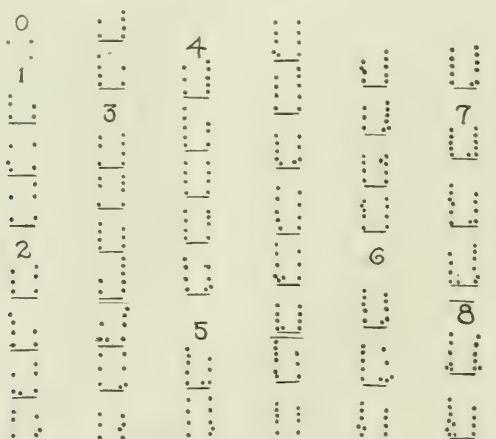


Fig. 1 Random bristle patterns showing some of the arrangements possible for the most common numbers of extra bristles. The numbers indicate the numbers of extra bristles in the patterns following.

#### THE ESTABLISHMENT OF THE RACE WITH EXTRA BRISTLES

The flies which gave rise to a race of *Drosophila ampelophila* having extra bristles on the thorax were found in a stock of wild flies that had been caught at Woods Hole in 1912 and bred in mass cultures for a year in Professor Morgan's laboratory. This stock had never been mixed by crossing, and in working

with the flies there has been found no reason to suspect accidental contamination. Flies with extra bristles were occasionally found in this stock. Their frequency is shown in table 1. Various other stocks were examined and extra bristles were found in smaller numbers.

TABLE 1  
*Extra bristles found in various stocks*

STOCK	EXTRA BRISTLES					TOTAL EXTRA BRISTLES PER CENT EX- TRA
	0	1	2	3	4	
Woods Hole wild.....	891	17	20	4	2	434.8
Falmouth wild.....	442	3				30.6
Edgewater wild.....	474	2				20.4
Cherry.....	266	2			1	31.1
Vermilion.....	163	2				21.2
Black.....	80					0
Orange.....	183					0
Peach.....	263					0
Bow.....	123					0
$\frac{1}{2}$ Balloon.....	189					0
White head.....	142					0
New York '12.....	3892	46	3			491.2

TABLE 2  
*Percentages of normals in successive inbred generations, selected for increase in bristles.*

GENERA- TION	NUMBER COUNTED	PER CENT NORMAL
1st	249	44.9
2nd	7139	10.0
3rd	2380	3.1
4th	1503	2.1
5th	1819	2.8
6th	2780	0.9
7th	5833	2.6
8th	2343	0.8
9th	1690	1.4
10th	2479	1.9
11th	2859	3.4

From the Woods Hole 1912 stock a male with one extra bristle was mated to a female with two extra bristles. Of their offspring 55.1 per cent had extra bristles. Thirty-three matings of first generation flies with extra bristles resulted in an  $F_2$  with 90 per cent extra bristles. The subsequent generations produced by inbreeding brothers and sisters in pairs gave higher proportions of *extras* (table 2). One might suppose that the occurrence of normals in the later generations indicated impurity; but in spite of being normal in regard to the group of bristles selected for observation, these flies frequently show extra bristles on other parts of the thorax, and, further, there will be presented evidence indicating that such normal appearing flies may produce all *extra* children; in other words, that other than genetic causes may prevent the development of potential extra bristles.

To test the stability of the *extra* race further, three mass cultures were started from the 4th and 6th inbred generations and have been carried along from bottle to bottle with no further selection. Occasional counts have been made of these cultures and it has been found that the extra bristles have been retained. Table 3 shows the per cent normals in mass counts. It is evident that there are more normals in some cases in these mass cultures than in the inbred lines, but these normals are more than likely potential extras whose greater frequency is due to the fact that in mass cultures there are larger numbers of flies which are smaller on account of the crowding. It will be demonstrated later that there is a relation between the size of the fly and the number of extra bristles. (See the discussion on the influence of environment).

## INHERITANCE

### 1. THE FACT OF INHERITANCE

That a race of flies constantly bearing extra bristles has been established, indicates in itself that the extra bristles are inherited. To show that this is not pseudo-inheritance, due to certain constant environmental conditions, a stock of wild flies caught in New York has been carried along under the same conditions as the mass cultures of the extra stock, and for a few generations this wild stock was bred in separate pairs as were the inbred selected lines. A small percentage of *extras* was found in this wild stock (tables 1 and 4) but this did not increase in time, nor did the progeny of separate pairs show any higher proportions of *extras*. Conclusive evidence that extra bristles are inherited and not primarily due to environment is afforded by crosses, which show also the mode of their inheritance.

### 2. THE KIND OF INHERITANCE

The wild stock New York 1912 was found to have fewer *extras* than any other wild stock examined and so from this stock normal flies were selected for several generations to attempt to reduce the percentage of *extras*. A preliminary set of four crosses

TABLE 3

*Occurrence of normals in three mass cultures*

MASS CULTURE	MONTH COUNTED	NUMBER COUNTED	PER CENT NORMAL	MASS CULTURE	BOTTLE NUMBER	NUMBER COUNTED	PER CENT NORMAL
105B	Oct.	67	0	105B <sub>1</sub>	10.18	150	2.6
	Nov.	107	0.9		12.50	432	4.4
	Dec.	391	2.5		12.12	952	1
	Jan.	251	5.1		12.28	152	1.3
	Feb.	84	3.5		1.13	193	8.8
	Mar.	138	4.3		1.22	90	2.2
	May	233	2.5		4.14	180	1.1
105B	Total	1304	3.6		12.90	1560	0.6
176	Total	1375	3.1		1.12	567	1
105B <sub>1</sub>	Total	394	2.5		4.14	167	1.8

TABLE 4

*Three generations of selection by pairs of normal New York stock to reduce the numbers of extra bristles and test the influence of mating by pairs on the numbers of bristles.*

GENERATION	BOTTLE NUMBER	FLIES COUNTED		
		Nos. extra bristles		
		0	1	2
1st	198	227	3	
2nd	214	265	2	
2nd	218	584	9	
2nd	219	290	5	
2nd	220	262	9	
3rd	241	220	1	
3rd	242	245	7	1
3rd	255		11	

between *extra* females from the first inbred generation, and normal males from the unselected New York 1912 stock, showed in general a dominance of the normal, although less than .5 per cent *extras* were found. These *extras* were always of very low grade, + 1 (one extra bristle), while the corresponding inbred generation of *extras* showed a range up to +7 (seven extra bristles) and a mode at +2. If extra bristles were supposed to be due to environmental conditions it would be difficult to account for their general disappearance in this generation. In the following generation, F<sub>2</sub>, the *extras* appear in proportions approximating the simple Mendelian ratio, although the normals are somewhat more numerous than expected. The *extras* that appear in F<sub>2</sub> include higher grades (table 5A).

A second set of crosses between *extras* and normals, after each race had been selected for a number of generations, was made. The *extras* used were from the 9th and 10th generations of inbred selection. In every cross the dominance of normal was

clear, although a few *extra* flies appeared in  $F_1$ . Among 1419  $F_1$  flies, 12 had one extra, 3 had two extra bristles. Sixty-five pairs of normal  $F_1$  flies were mated. The total counts for their children are 8421 normal, 2499 extra, which give a ratio of 3.3:1. (table 5B). As in the previous crosses there is a practical incomplete dominance in  $F_1$ , whatever theoretical explanation may be given, and in  $F_2$  the numbers of extras are a little too low, yet there can be no question that this is clear evidence of the existence of a Mendelian factor that influences the number of bristles.

To test for sex linkage, five of the above crosses were made with *extra* males and three were made with *extra* females. In

TABLE 5

*Giving the results of crosses between extra bristled parents and normals from wild stock (New York '12)*

A—Extra bristled parents from the second inbred generation

BOTTLE NO.	PARENTS ♂: ♀	F <sub>1</sub>			F <sub>2</sub>									RATIO	
		Normal	Extra		No. of matings	Number of extra bristles									
			1	2		1	2	3	4	5	6	7	8	9	
20	0:2	173	3	—	2	172	5	10	1						10.7:1
21	0:2	210	—	—	4	463	47	60	11	2					3.8:1
22	0:2	255	1	—	2	466	63	46	14						3.8:1
IX	0:3	197	—	—	3	423	58	49	11	2					3.5:1
Total		835	4	—	11	1524	173	165	37	4					4.0:1

B—Extra bristled parents from the eighth inbred generation

316	3:0	156	3												
311	3:0	301	2	—	8	994	89	86	52	23	10	2	1		3.5:1
314	2:0	40	—	—	5	762	67	61	46	34	27	3	2	1	3.1:1
312	6:0	93	2	—	15	1383	169	102	93	51	18	8	2		2.9:1
313	6:0	189	2	1	15	1487	138	121	90	38	20	5	4		3.5:1
304	0:3	5	—	—	1	195	22	14	8	3	2	1			3.9:1
305	0:2	432	3	2	9	1646	166	145	111	45	31	9	4		3.1:1
307	0:6	203	—	—	11	1954	192	166	109	64	33	6	5		3.3:1
Total		1419	12	3	64	8421	843	695	509	258	141	34	18	1	3.3:1
Totals of all crosses		2254	16	3	75	9945	1016	860	546	262	141	34	18	1	3.4:1

both types of crosses the children showed dominance of the normal, and all grandchildren gave the same general ratios of normal to *extra*. Moreover, the distributions of these  $F_2$  *extras* were similar in the two types of crosses (table 9 and figure 4). This is enough to indicate that there is no sex linkage involved.

### 3. MODIFICATION OF THE DISTRIBUTION OF EXTRACTED EXTRAS

In comparing the distribution of the extra bristles in the  $F_2$  of the above crosses with that of the extra bristles in the selected race of the corresponding generations, a marked difference is observed. This is shown in figure 2. In these curves the males and females have been put together and the normals occurring in the inbred have been omitted, since corresponding normal appearing flies in the  $F_2$  of the crosses could not be separated from the genetically normal flies. The data have been plotted on the basis of 500 in each curve, to facilitate comparison. In general the distribution of the *extras* appearing in the  $F_2$  of a cross is lower than the distribution of the corresponding generation of uncrossed *extras*. The mode of the extracted extra bristles from the first cross is at + 1, although the frequency of + 2 is nearly as great; the mode of the second selected generation is at + 2. In the later crosses, the mode of the extracted extra bristles is at + 1, and the mode of the tenth selected generation is at + 3.

With these curves in mind it will be well to compare the extracted *extras* from flies crossed before continued selection, with the extracted *extras* from flies crossed after selection. It is readily seen that the extracted curve from the later crosses is strikingly higher than that from the first crosses. The range is much higher; but the mode is still at + 1, even more prominently so than in the first case. The standard deviation in the first set of extracted *extras* is  $.695 \pm .017$ , that of the corresponding inbred generation is  $.863 \pm .005$ . In the later crosses standard deviations are as follows: extracted *extra*,  $1.329 \pm .018$ , inbred,  $1.402 \pm .021$  (table 6). It appears to be a very definite fact that the modification of the distribution of extra bristles by

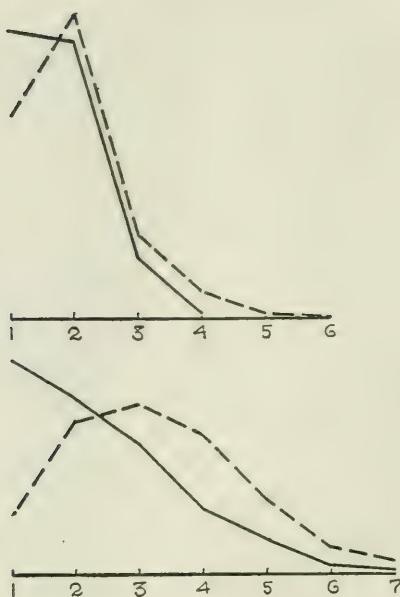


Fig. 2 Comparisons of the distributions of inbred and extracted extra bristles in corresponding generations. Upper curves, selected inbreds of the second generation (broken line) and extracted *extras* before selection (solid line); lower curves, selected inbreds of the tenth generation (broken line), and extracted *extras* of the corresponding generation after selection (solid line).

crossing becomes more marked when parents are selected for several generations before being crossed, yet this is not accompanied by any increase in variability.

#### 4. SUMMARY

It has been shown that the normal number of four bristles is dominant to the extra bristled condition; that the simple Mendelian ratio of 3:1 is closely approximated; that no sex linkage is involved. Further, it has been shown that the flies having extra bristles in the  $F_2$  of a cross have a distribution lower than that of the selected flies of the corresponding generation, and that this difference is more marked when extra bristled flies are crossed that have been selected for nine generations

TABLE 6

*To compare the means and the variability of extra bristles that appear after a cross with those in corresponding generations of the extra race.*

	MEANS	NUMBERS	STANDARD DEVIATION
Extracted Extras (Gen. 2).....	1.665	377	0.695±0.017
Inbred Extras (Gen. 2).....	1.921	6418	0.863±0.005
Extracted Extras (Gen. 10).....	2.335	2499	1.329±0.018
Inbred Extras (Gen. 10).....	3.229	981	1.402±0.021

than when flies of the first selected generation are crossed. The variability of the extracted extra bristles is slightly less than that of the corresponding inbred generation.

### 5. DISCUSSION

The interpretation that seems most simple to apply to the above facts is that there is a positive restricting factor present in the wild fly that prevents more than four bristles from developing. Although the *extra* race has something added somatically to it, genetically it has lost a restricting factor. Now this condition of extra bristles seems not to be phylogenetically new, as studies on the chaetotaxy of related flies suggest. The mechanism that is most concerned must have appeared when *Drosophila ampelophila* arose, or before, so the extra bristles are not formed by the origin of a new mechanism, but rather by the removal of the restriction of the more recent mechanism.

### THE SELECTION PROBLEM

The main problem to which the preceding experiments have been contributory, has been the attempt to throw some light on the question of selection as a formative process in evolution. The attempt has been made to increase the numbers of thoracic bristles as much as possible by selecting and inbreeding. Accordingly, after the first pair that came from wild stock, high grade brothers have been mated to high grade sisters. In the following account the first eleven generations of this process will be described.

1. DO HIGHER GRADE PARENTS PRODUCE HIGHER GRADE CHILDREN?

To show what basis there may be for selection to progress, comparisons have been made of children from high and low parents from the same grandparents. All the matings in  $F_1$  can be used for this purpose, as all the parents were sibs. Curves showing the 35  $F_2$  fraternities were plotted, but they were too numerous for publication. The children from parents of like grades have been grouped together. Table 7 shows the averages of the males and females, the numbers of flies and of families involved in the various types of matings. No increase in the means is found that directly corresponds to the increase in parental values. However, in grouping together children from parents with one or two extra, and comparing them with children from parents with three or four extra, the higher group has, in general, higher means. The low means of the one 4:4 mating involves too few individuals to make a serious exception. In the second generation there seems to be a basis for some effective selection.

Similar comparisons can be made in the later generations. Seven families in  $F_4$  came from the same grandparents. Thirteen of the parents were + 5 and one, + 6. Here is a case where the parents are closely alike and one would expect the children in the different families to be closely alike. The curves and the

TABLE 7

*To show the relationship between the grades of the parents and the means of their children in the second selected generation*

GRADES OF PARENTS	NUMBER OF MATINGS	MEANS		NUMBERS	
		Male	Female	Male	Female
1:1	4	1.70	2.01	397	418
1:2	3	1.45	1.88	367	375
2:2	17	1.42	1.84	2061	2204
3:3	3	2.05	2.46	171	229
3:4	2	1.94	2.39	207	220
4:4	1	1.61	1.98	33	41

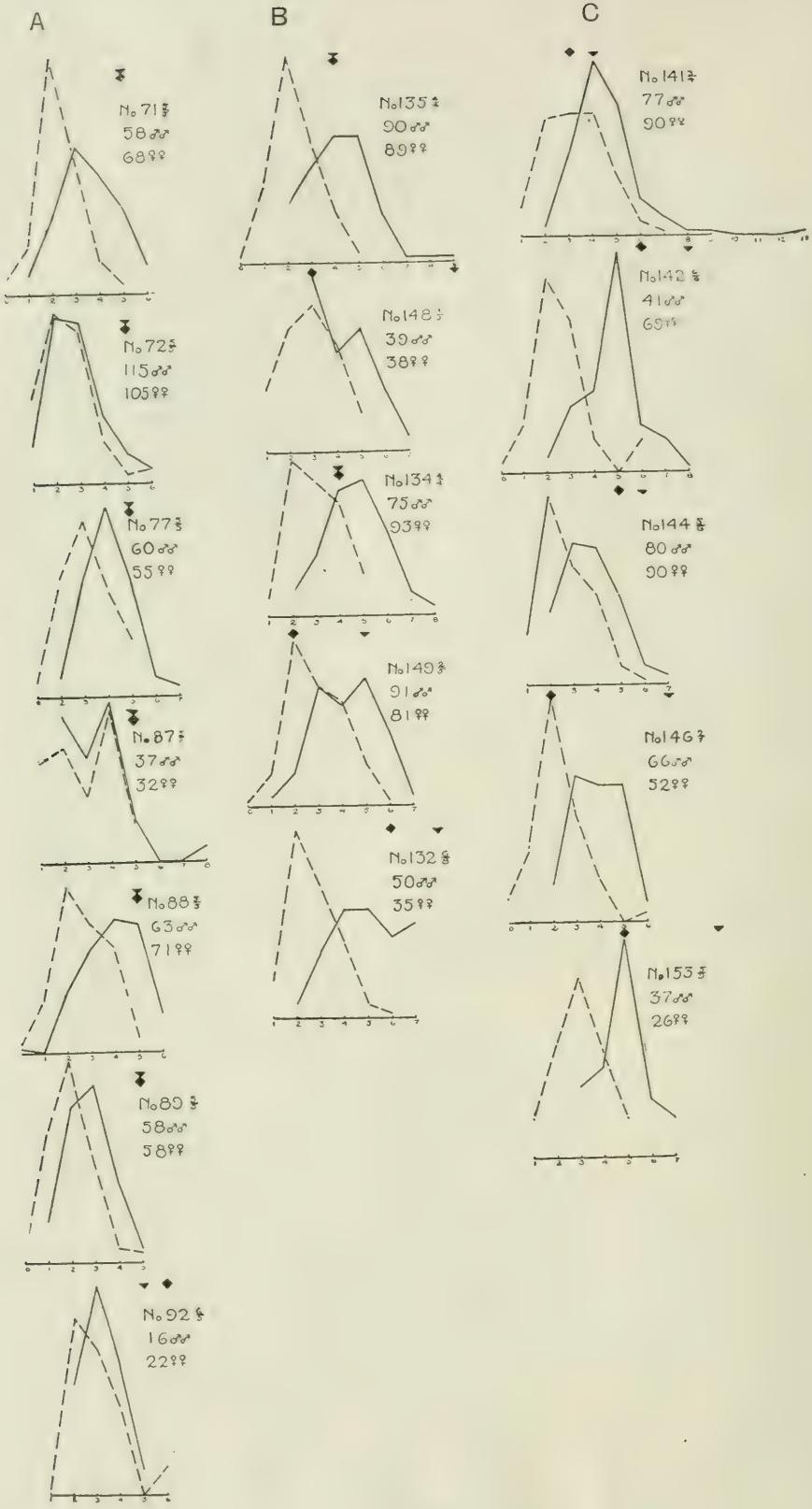
averages (fig. 3 and table 8) show that this is not the case. The averages for females range from +2.83 to +4.05. This set of families gives some idea as to how much variation is possible when the grandparents are brother and sister and the parents all sibs of the same grade.

In F<sub>6</sub> are two sets of cousins. In one group are five families (fig. 3B), grandchildren of 115, with parents of various grades. Considering the female averages (table 8B) it is seen that the parental sums of 7, 8a, and 12 give similar filial averages, whereas the parental sums of 8b and 14 give higher averages. Considering the males, parental values of 7, 8b, and 12 give similar

TABLE 8

*Six groups of cousins. In each group the parents are sibs; grades of parents and means of offspring arranged according to the sums of the parents' grades. See figure 3*

BOT. NO.	PARENTS		SUM OF PARENTS	MEANS OF CHILDREN		NOS. OF CHILDREN	BOT. NO.	PARENTS		SUM OF PARENTS	MEANS OF CHILDREN		NOS. OF CHILDREN
	♂	♀		♂	♀			♂	♀		♂	♀	
A children in F <sub>4</sub>													
71	5	5	10	2.29	3.53	58	68	177	4	4	8	2.61	3.90
72	5	5	10	2.46	2.83	115	105	166	5	6	11	3.19	5.16
77	5	5	10	3.15	4.05	60	55	178	5	9	14a	3.00	4.98
87	5	5	10	2.81	3.37	37	32	180	6	8	14b	2.85	5.19
88	5	5	10	2.66	3.91	63	71						
89	5	5	10	1.98	2.74	58	58						
92	6	5	11	2.87	3.13	16	22	289	5	5	10	3.28	4.40
								284	6	5	11	2.75	3.88
B children in F <sub>6</sub>													
149	2	5	7	2.91	4.17	91	81	290	6	6	12a	3.25	4.57
135	4	4	8a	2.36	4.15	90	89				12b	3.40	5.46
134	4	4	8b	3.01	4.66	75	93						
148	3	9	12	2.95	4.23	39	38	413	5	5	10	3.41	4.61
132	6	8	14	2.78	4.97	50	35	411	6	5	11	3.98	5.73
								329	6	6	12a	1.76	2.72
C children in F <sub>6</sub>													
141	3	4	7	3.27	4.54	77	90				12b	3.20	4.54
155	4	4	8	3.00	4.63	11	11						
146	2	7	9	2.22	3.90	66	52						
144	5	6	11	2.67	3.71	80	98						
142	6	8	14a	2.60	4.73	41	69						
153	5	9	14b	2.97	4.76	37	26						



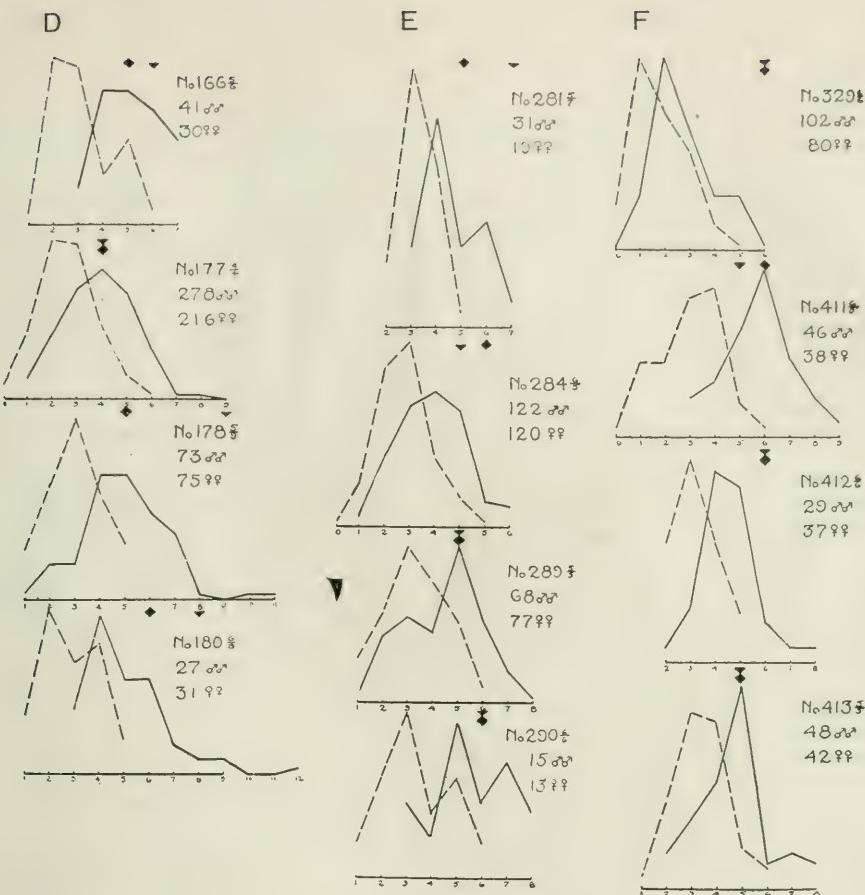


Fig. 3 Six groups of cousins; in each group the parents are sibs. Solid lines are females, broken lines males. The parents are indicated by squares (males) and triangles (females) above the curves of their children. The fractions following the numbers of the mating indicate the grades of the parents. The numbers of males and females involved in the curves are given at one side. A, grandchildren of no. 56, in the 4th generation; B, grandchildren of 115, in the 6th generation; C, grandchildren of 116, in the 6th generation; D, grandchildren of 143, in the 7th generation; E, grandchildren of 246, in the 10th generation; F, grandchildren of 274, in the 11th generation.

averages, 14 a little lower, and 8a much lower. In the second group of families in  $F_6$  (fig. 3C and table 8C) female averages from parental sums 7, 8, 14a, and 14b are similar, whereas those from sums 9 and 11 are a whole bristle lower. The male averages for parental sums 7, 8 and 14b are similar while those from 11 and 14a are lower, and from sum 9 a whole bristle lower.

In  $F_7$  is a set of cousins consisting of four families (table 8D and figure 3D). In this group the low parental sum has the low male and female averages, whereas sums 11 and 14a and 14b are much alike. In  $F_{10}$  there is a group of cousins in four families (table 8E and fig. 3E). The female average for parental sum 12b is considerably larger than that for the sum 10, yet the female average for the sum 12a is about equal to that for the sum 10. Parental sum 11 has the lowest male and female averages. In  $F_{11}$  a group of four families (table 8 F and fig. 3F) shows averages for parental sum 11 that are above averages for 10 and 12b. Parental sum 12a has averages much below any of the other families. This study of averages must be followed by careful observation of the figures referred to above, which show the frequency distributions of each of the families that has been considered. These curves have been plotted so as to enclose in each polygon similar areas, to facilitate comparisons. The actual numbers of flies are given both in the tables and the figures.

Further data bearing on the question of the relation between the grades of parents and children is to be found in the series of curves representing inbred lines through single matings (table 11 and fig. 6). One case must be especially emphasized. The last generation, No. 216, in the series beginning with No. 18 (fig. 6 A) was produced by flies with no extra bristles. This generation which follows seven selections has a higher distribution than the preceding ones which were from high grade parents. There are no normals from these normal parents, and no females with less than two extra bristles. This extreme case is in accord with all that has been found in regard to the independence of the parental and filial grades. This is further borne out by these inbred lines in the comparison of parents and children in successive generations.

So the general conclusion seems to stand, that after the first few generations there is no close relationship between the grades of the parents chosen and the grades of the children. This appears to mean that in regard to extra bristles the flies in later generations do not differ genetically from each other; that in the later generations the variability in the number of extra bristles is due mainly, if not entirely, to conditions outside the germplasm. A further test of this would be to cross with normals flies with many extra and flies with few extra bristles and compare the results in the second generations. If the difference between the many and the few extra bristles be due to genetic factors, this would be shown in the  $F_2$  of the crosses. Data is given that shows the distributions of extracted extra bristled flies from crosses of high and low *extras* with normals of a different race. To avoid any complication that might arise from not considering sexual differences, and at the same time to find evidence in regard to sex linkage, four types of crosses with wild flies have been made: high and low males, high and low females. In table 9 the data are arranged to facilitate comparisons between males and females of like grades, but by comparing alternate lines the relations between high and low grades are clear. The ratios and the distributions are practically alike in all cases (fig. 4). In order to make the curve for high males, including over 800 individuals, more easily comparable with the others, these data were plotted on the basis of 600.

In conclusion, comparisons of families in the second generation of inbreeding show a tendency for higher parents to produce higher children, but this is not found in the sixth or subsequent generations; in single inbred lines there is found singular independence of the parental and filial grades, seemingly normal parents being able to produce all *extra* children; by the analysis of crosses it is found in the ninth and tenth generations that, as should be expected from the foregoing statements, high and low bristle grades are genetically indistinguishable; that the variability found in the late generations is apparently not due to genetic factors.

TABLE 9

Showing that low and high grades of extra bristles give the same results when crossed with wild normals; also that reciprocal crosses show that no sex linkage is involved

P <sub>1</sub>	F <sub>1</sub>		F <sub>2</sub>									RATIO NORMAL TO EXTRA		
	normal	1	2	normal	1	2	3	4	5	6	7	8		
Low ♂♂ × Wild ♀♀ . . . .	341	2		1756	156	147	98	57	37	5	3		1	3.4:1
Low ♀♀ × Wild ♂♂ . . . .	437	3	2	1841	188	159	119	48	33	10	4			2.8:1
High ♂♂ × Wild ♀♀ . . . .	282	4	1	2870	307	223	183	89	38	13	4			3.3:1
High ♀♀ × Wild ♂♂ . . . .	203			1954	192	166	109	64	33	6	5			3.3:1

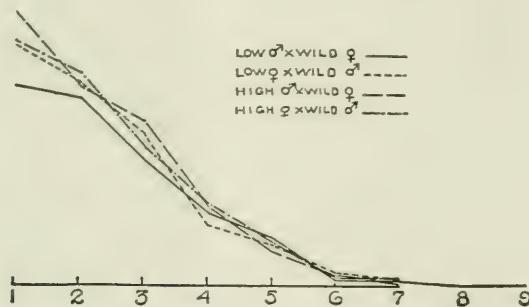


Fig. 4 Extracted extra bristles appearing in F<sub>2</sub> of reciprocal crosses of high and low grades of extra bristles with normal wild, showing that there is no factorial difference between the high and low grade *extras* crossed.

## 2. RESULTS OF SELECTION

### a. Total generations

On the basis of the conclusions just reached, we should expect to find selection effective in the first few generations and later, ineffective. Data will be presented to show the results of selection in the form of curves for total generations and for single lines. Owing to the difficulty of obtaining high grade females that were unquestionably virgin, the most rigid selection was not always possible, so that even in the later generations some matings were made of low parents. In order to avoid the possibility of hiding a real increase in the offspring from high grade parents by including the offspring from low grade parents,

the children having either parent below certain grades were omitted. Along with these were excluded all flies whose grandparents or more remote ancestors had been excluded. This reduced the numbers in the total generations very considerably, and this is especially so in the latest generations. In these resulting curves the parents of each generation are included in the curve of the preceding generation. In figure 5 the curves have been plotted in such a way as to include similar areas, although representing different numbers of individuals. For this reason the numbers of males and females included are put down beside the curves. The solid line represents females; the broken line, males. The small curves between the larger ones in each case represent the parents selected from the generation above, to produce the generation below.

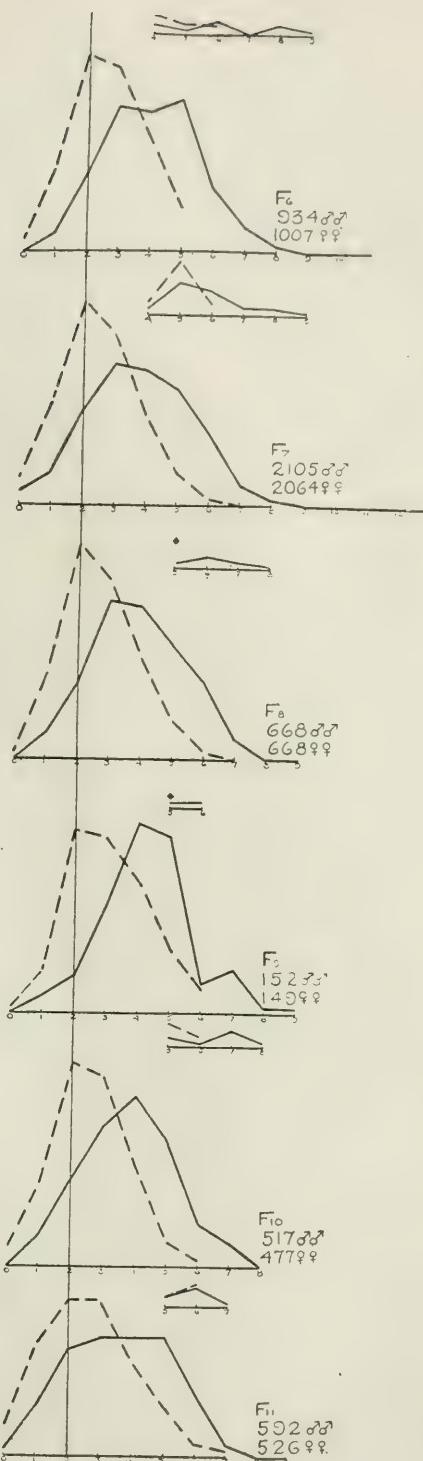
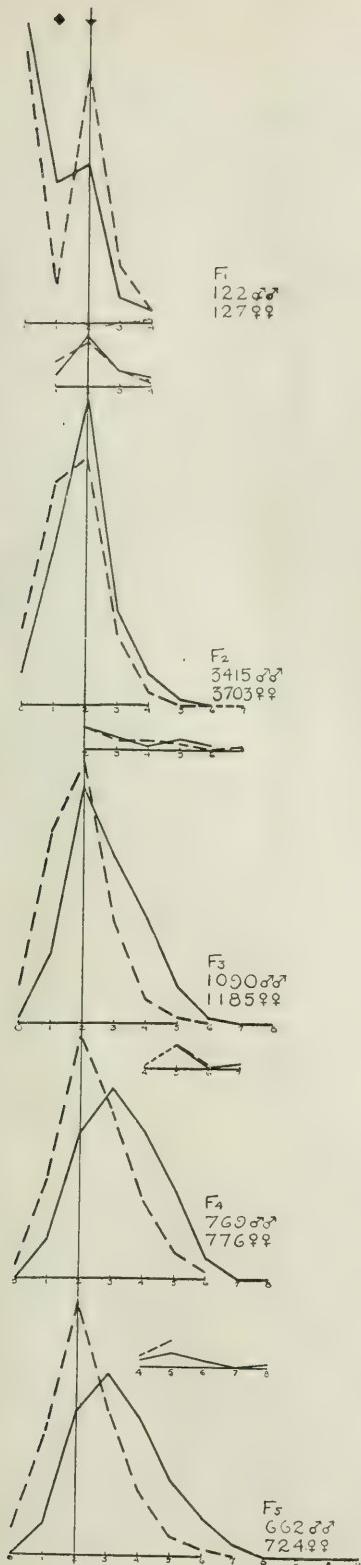
*Discussion of the curves for total generations.* The original parents selected from wild stock were a male with one extra bristle (+1) and a female with two extra bristles (+2). Their children are represented in the first pair of curves. The large number of normal flies in this generation 44.90 per cent is due to the fact that the mother was not virgin. Disregarding these, the male and female modes are at +2. In the following generation the modes remain at +2 but the upper limit of the range goes up from +4 to +7. The flies chosen as parents from this generation, range from +2 to +7. In the third generation the modes are still at +2 but the male curve has moved a little higher and the female curve is markedly above the male. This is clearly seen by comparing the areas of the polygons above the vertical line drawn at +2. The flies chosen from this generation range from +4 to +7. In the fourth generation the female mode is raised to +3 and a clear advance is shown by the whole curve. Although the male mode is still at +2, the proportion of +1 males has decreased and the proportions of +3 and +4 have strongly increased. In the fifth generation the male curve has increased its range over the preceding generation but as a whole has fallen back a very little. The female curve has a much increased range, and a slight sag above the mode, otherwise no change. In the sixth generation

the male mode still holds to +2 but the proportion of +3 flies has grown to nearly equal the mode. The proportions of +4 and +5 flies have also increased although +5 is the limit. The female mode is actually at +5 but the expected mode is easily seen to be at +4. So far the progress and increase has been steady and unquestionable. The following five generations do not show any such advance. In generations seven, eight, nine, and ten the male mode remains at +2 and the proportion of +3 flies is very slightly less. In the eleventh generation the +2 and +3 males are equal, however the proportion of +1 flies has greatly increased in this generation, so this does not mean any general advance. The female curves have modes at +3 in the 7th and 8th generations, with +3 nearly as high. In the last 3 generations the female mode is at 4 and the curves are in general of the same kind.

To show what parents were used in the different generations more clearly than can be done by the curves, their distributions are shown in table 10. The averages of the parents are given in two columns at the left and the averages of their children are in two columns towards the right. This table gives a clear summary of the progress of selection. The averages of the children, males and females, show increase up to the sixth generation, when minor fluctuations appear seemingly without any significant change.

If the variability in the last five generations be amenable to selection, a gradual decrease in the standard deviation would be expected, even though some hypothetical physiological limit prevented the means and modes from advancing as before.

Fig. 5 Eleven generations of selection. Original parents indicated by a square (father) and triangle (mother) above  $F_1$  broken lines, males; solid lines females. The flies chosen as parents are arranged in small curves above the curves of their children. In generations 8 and 9 the male parents were all grade 5 and are represented by a square the proper distance from the base line. A finely dotted line means males and females together. The curves are plotted in such a way as to include like areas, so the numbers of individuals included are given at the side of each curve. The upper limits are in some cases drawn on the base line to show that such extremes appeared, although in too small numbers to form a whole unit in plotting.



But the standard deviations show a gradual increase while selection is effective according to the curves and the means, and when selection has seemingly become ineffective, the standard deviations fluctuate, but do not show a gradual decrease.

### b. Single lines

The most immediate facts are presented in the form of the history of five inbred lines through single matings, as shown by curves in figure 6, A, B, C, D, E. The grades of the parents selected from each generation are indicated below the curve of their own fraternity and above the curves representing their offspring. Squares are the males; triangles are the females. Four of the lines start from the same pair of first generation flies. The first two generations which are common ancestors of all four lines, are represented in only one set of curves. The parents of families Nos. 87, 71 and 88 are all sibs in family No. 56. The parents of family No. 116 come from family No. 88. The line headed by family No. 17 starts with a different pair of first generation flies. As the history told by these curves is clear it does not seem necessary to discuss each set of curves in detail as has been done for the total generations. In Table 11, A, B, C, D, E, the means for these curves are given. These curves give the clearest picture of the basic facts. Clear progress is shown in the first five or six generations. In each line are differences counter-balanced in the total generations, yet in no case do these differences modify the general conclusion drawn from the study of total generations.

### 3. SUMMARY

In brief, selection appears to have made advances for six generations. When large enough numbers are observed to afford a counter-balancing of the fluctuations of individual families, the advance is steady. That this increase is really genetic is shown by the increase in the distribution of *extras* extracted from a cross of long selected flies, over the distribution of *extras* extracted from a cross of unselected flies. In later generations

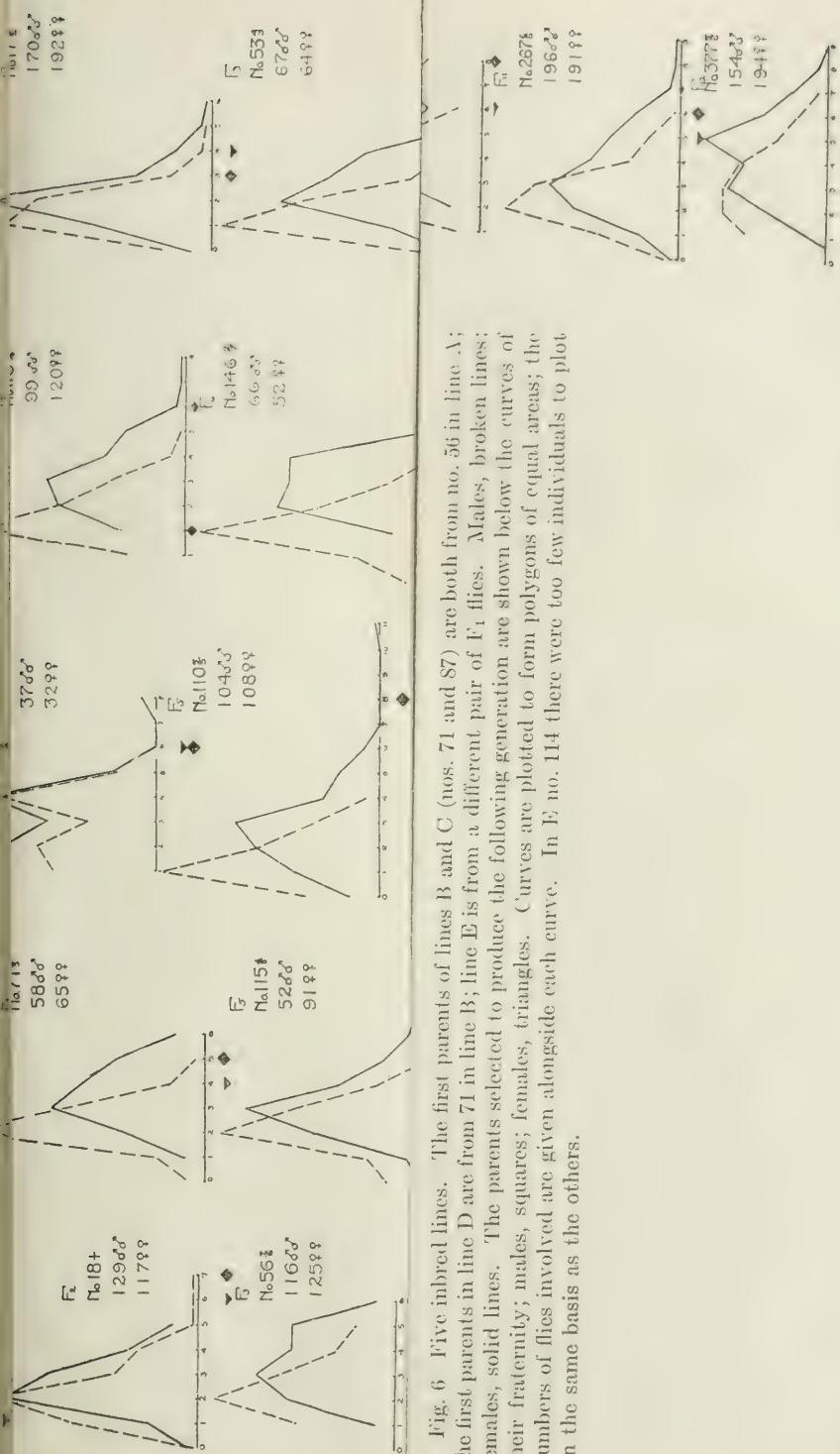


Fig. 6 Five inbred lines. The first parents of lines B and C (nos. 71 and 87) are both from no. 56 in line A; the first parents in line D are from 71 in line B; line E is from a different pair of  $F_1$  flies. Males, broken lines; females, solid lines. The parents selected to produce the following generation are shown below the curves of their fraternity; males, squares; females, triangles. Curves are plotted to form polygons of equal areas; the numbers of flies involved are given alongside each curve. In E no. 114 there were too few individuals to plot on the same basis as the others.

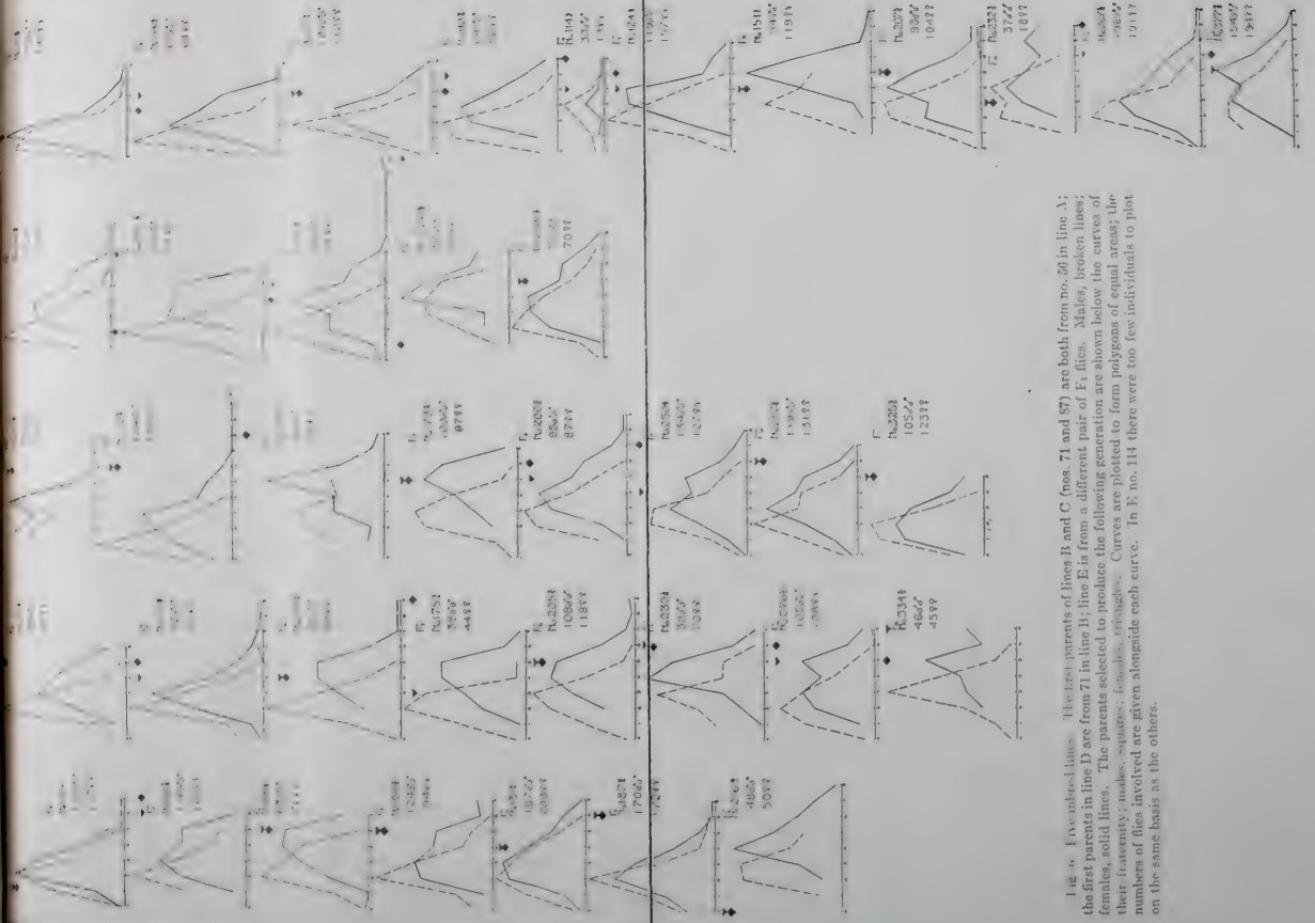


FIG. 6.—Five related lines. The test parents of lines B and C (nos. 71 and 87) are both from no. 56 in line A; the first parents in line D are from 71 in line B; line E is from a different pair of F<sub>1</sub> flies. Males, broken lines; females, solid lines. The parents selected to produce the following generation are shown below the curves of their identity; males, squares; females, diamonds. Curves are plotted to form polygons of equal areas; the numbers of flies involved are given alongside each curve. In F no. 114 there were too few individuals to plot on the same basis as the others.



TABLE 10

Showing the distributions of the parents selected and the averages and variability of their children in the eleven generations of inbreeding and selection.

Generation	PARENTS									OFFSPRING													
	Distribution according to no. of extra bristles									Means		Nos. of individuals		Modes		Means				Standard deviations			
	1	2	3	4	5	6	7	8	9	Males	Females	Generation	Males	Females	Males	Females	Males	Females	Males	Females			
F <sub>1</sub>	12	38	10	4	—	—	—	—	—	1.96	2.21	F <sub>2</sub>	3415	3703	2	2	1.494 ± .010	1.453 ± .009	0.912 ± .007	0.829 ± .006			
F <sub>2</sub>	—	14	7	4	5	1	1	—	—	3.253	3.18	F <sub>3</sub>	1090	1185	2	2	1.916 ± .021	2.708 ± .022	1.035 ± .014	1.201 ± .017			
F <sub>3</sub>	—	—	2	14	1	1	—	—	—	5.00	5.11	F <sub>4</sub>	769	776	2	2	2.219 ± .027	2.925 ± .031	1.122 ± .019	1.284 ± .022			
F <sub>4</sub>	—	—	5	12	2	—	1	—	—	4.50	5.10	F <sub>5</sub>	662	724	2	3	2.300 ± .030	3.480 ± .035	1.147 ± .021	1.415 ± .025			
F <sub>5</sub>	—	—	—	9	4	7	—	3	14	75	6.16	F <sub>6</sub>	934	1007	2	5	2.703 ± .026	4.055 ± .036	1.164 ± .018	1.729 ± .026			
F <sub>6</sub>	—	—	—	6	27	10	2	2	14	96	5.79	F <sub>7</sub>	2105	2064	2	3	2.507 ± .015	3.694 ± .023	1.025 ± .010	1.530 ± .016			
F <sub>7</sub>	—	—	—	—	11	4	2	1	—	5.00	6.22	F <sub>8</sub>	668	668	2	4	2.682 ± .031	3.878 ± .037	1.186 ± .023	1.407 ± .027			
F <sub>8</sub>	—	—	—	—	6	2	—	—	—	5.00	5.50	F <sub>9</sub>	152	149	2	3	3.065 ± .069	4.382 ± .083	1.276 ± .049	1.504 ± .059			
F <sub>9</sub>	—	—	—	—	10	4	5	1	—	5.30	6.40	F <sub>10</sub>	517	477	2	4	2.615 ± .035	3.800 ± .045	1.172 ± .025	1.469 ± .032			
F <sub>10</sub>	—	—	—	—	—	6	13	1	—	5.70	5.80	F <sub>11</sub>	592	526	2	4	2.050 ± .044	3.831 ± .053	1.589 ± .031	1.801 ± .037			

TABLE 11

Grades of parents and means of children in five inbred lines (see figure 6)

GEN.	BOT. NO.	PARENTS		MEANS OF CHILDREN		NOS. OF CHILDREN	GEN.	BOT. NO.	PARENTS		MEANS OF CHILDREN		NOS. OF CHILDREN				
		♂	♀	♂	♀				♂	♀	♂	♀					
A	2	17	2	2	1.53	2.03	170	192	C	4*	71	5	5	2.29	3.53	58	68
	3	53	4	3	1.57	2.57	67	69		5	115	4	5	2.27	3.35	52	91
	4	67	4	4	2.10	2.76	101	110		6	135	4	4	2.36	4.15	90	89
	5	98	5	4	1.87	3.14	64	80		7	175	3	9	2.18	3.93	38	44
	6	114	4	6	2.42	3.16	33	13		8	205	5	5	2.62	4.27	108	118
	7	124	4	5	2.51	3.57	119	157		9	239	6	6	2.84	4.25	38	70
	8	151	4	4	3.32	4.98	94	119	D	4*	87	5	5	2.81	3.37	37	32
	9	207	5	5	2.73	3.98	93	104		5	110	5	5	2.44	3.75	105	108
	10	232	3	3	3.41	5.06	37	18		10	276	5	6	2.44	3.75	105	108
	11	267	5	8	2.49	3.41	196	191		11	334	7	5	3.07	4.60	46	45
B	12	377	5	6	2.95	4.26	149	194		12	377	5	8	2.95	4.45	46	55
	2	18	1	1	2.23	2.59	129	117		7	173	5	5	2.83	4.59	109	97
	3	56	7	6	2.50	3.44	116	125		8	206	5	6	2.91	4.26	96	87
	4	88	5	5	2.67	3.91	63	71		9	250	4	7	2.67	3.73	134	127
	5	106	5	5	2.47	3.57	124	94		10	287	6	6	2.47	3.36	119	131
	6	131	4	4	2.50	3.20	187	203		11	325	5	5	1.65	2.28	105	123
	7	187	6	6	2.01	2.42	170	172		12	377	5	8	2.95	4.45	46	55
	8	216	0	0	2.98	4.56	48	50	E	5†	116	4	4	2.57	4.00	99	120

\* Preceding generations are those given under B.

† Preceding generations are those given under C.

(after the 6th) as far as the eleventh, no further advance has been detected. This is what the comparisons of different families from the same grandparents, or successive generations of inbred lines, had led one to expect. In the second generation there is a tendency for parents of higher grades to produce children of higher grade, while this is not the case in the later generations where high and low variates seem to have the same genetic potentiality, and in so far as this is true, the variability must be explained by extra germinal causes.

#### THE ROLE OF THE ENVIRONMENT

##### 1. INFLUENCE OF FOOD

In seeking causes for variability in the number of extra bristles, studies have been made on certain factors of the environment, namely, food and temperature. It was early observed that if any normal flies came from a bottle of inbred selected flies they were apt not to appear among the first flies to hatch in that bottle. Also it became evident that more of the high grades did appear among the first flies hatched, than among the last. To make this clear the data was arranged to show the distributions of the flies counted on successive days from individual bottles. In many cases it was clearly shown that successive days showed lower and lower distributions of extra bristles. In other cases there was no such decline. However, in all cases the highest flies appeared among the first flies drawn off. That the falling off in bristle numbers is not due to any differences in the fertilized eggs is shown by the fact that whenever there is found such a decline in bristle numbers at the end of a bottle, the first flies hatched from the next bottle into which the same parents had been placed, show bristle numbers as high as those found at first in the preceding bottle. This was found to hold good for all cases, even though the parents were moved into three or more successive bottles. When the changes from bottle to bottle were frequent there was less falling off at the end of a bottle. Since the facts are so clear it seems needless to publish a large number of examples. Table 12 gives 3 ex-

amples from the 50 families so arranged. In mating No. 78, there was no falling off at the end of first bottle, but the second bottle shows very clear falling off. There is found, then, a certain relation between the time of hatching and the number of extra bristles, due to conditions external to the germplasm, namely, that the last flies from a bottle may be of lower grade

TABLE 12

*Daily counts of progeny from the same parents in successive bottles, showing that when the bristle numbers are lower at the end of a bottle, the high grades are regained at the beginning of the next bottle.*

MAT- ING NO.	BOT- TLE	MO.	DAY	0 1 2 3 4 5 6 7 8								MAT- ING NO.	BOT- TLE	MO.	DAY	0 1 2 3 4 5 6 7 8									
				0	1	2	3	4	5	6	7					0	1	2	3	4	5	6	7	8	
131	1st	10	14	1	1	2	4	5					145	1st	10	22		2	5	3	3	1		2	
		15		4	18	9	7	3							23		1	6	5	5	3	1			
		16	1	1	6	3	2	1							24		1	4	9	9	2	1			
		17		4	6	7	3								27	2	8	14	8	3	2				
		20	5	9	12	2									28	2	3	7	4	2					
	2d	22		1	1										30		10	5	4						
		22	8	8	26	14	15	7	2	1					31		2	3						1	
		23	1	2	3	3									30			2	4	1	1				
		24		4	7	6	3								31		2	2	5	4	3	2			
		27	4	16	29	15	2						11	2d	1			3	1	1	1	3			
3d	2d	24		3	5	5	8	8							3	1	4	14	9						
		27		2	9	20	21	6	1	1					6		1	4	1	1	1	1			
		28		4	2	1																			
106	1st	9	23	1	4	10	2	2	1	1			78	1st	9	8		3	26	15	18	3			
		25	1	3	15	8	4								4	1	5	12	11	5					
		26		3	7	3									5		6	16	4	1					
		30	2	1	5	2									6		3	6	5	2					
	2d	26		2	4	6	5	3	2						7		2	19	12	2	1				
		28		7	15	13	12	1	1						8		2	9	2	2					
		30		3	3	2	3								9		10	8	9	6					
		10	4	2	4	17	7								10		6	3	3						
		5	2	1	6	1									11		4	2	5	2					
3d	2d	4		3			1		1						12		1	2	2	2					
															15	1	3	7	3	1					
															18		2	1	1						
															19		1	2							

than the first ones, and when this is true, the higher grades reappear in the next bottle

It is common knowledge that the last flies from a bottle are apt to be smaller than the first ones hatched. This decrease in the sizes of the flies depends probably entirely upon the amount of food the flies eat while larvae; this amount in turn depends

upon the condition of the food and the environment. The similarity in the occurrence of decreased bristles and decreased size at the last of a bottle led to the idea that there might be some relation between the two phenomena. To see if smaller flies are apt to have fewer bristles, the total body lengths of 1400 random flies have been measured and their bristles counted. The data have been arranged in correlation tables. From table 13 one finds that up to a certain body size there appears a sort of correlation, in that the smaller the fly, the fewer the extra bristles it is apt to show. Above this size (grade 500) there

TABLE 13

*Males and females. Correlation between size and numbers of extra bristles*

SIZE GRADES	NOS. OF EXTRA BRISTLES								
	0	1	2	3	4	5	6	7	8
300	1								
325		1							
350	2	5		1	1				
375	2	3	7	1	1				
400	2	3	13	2	1				
425	2	10	24	6	9	1			
450	4	35	73	52	20	3		1	
475	5	39	94	76	39	14	6	1	
500	2	15	76	90	74	41	19	4	1
525			11	18	32	38	17	5	1
550		1	7	13	20	16	15	1	2
575		1	5	3	6	5	5	1	
600			1	1	1	3	3		

appears to be no constant relation between the increase in size and the numbers of extra bristles. However, it will be noted that above this size there are no normals. It has been concluded that the factor controlling extra bristles is not sex linked; however, nearly all the curves of extra bristled flies that have been presented, have shown that the males have fewer bristles than the females. Correlation tables were made for males and females separately (tables 14, 15). These show strikingly enough, that the males have fewer bristles than the females and are smaller, no male being above grade 500. This does not mean

that a male may never be larger than this, but the comparison with the females is accurate since males and females were both used in each group of flies that was measured.

In the mass cultures of extra bristled flies the bristle numbers are somewhat lower and the number of normals higher than in the inbred lines, but the flies themselves being raised in larger numbers and so cramped in close together are much smaller

TABLE 14

*Females. Correlation between size and number of extra bristles*

SIZE GRADE	NO. EXTRA BRISTLES								
	0	1	2	3	4	5	6	7	8
350	1								
375									
400									
425		3							
450	3	5	1	2					
475	2	7	6	3	2	2			
500	4	19	20	16	5	4	1		
525		5	1	9	9	4	1		
550	1	5	2	5	5	2	1	1	
575	1	2	1	3	1	1	1		
600		1			2	1			

TABLE 15

*Males. Correction between size and numbers of extra bristles.*

SIZE GRADE	NO. EXTRA BRISTLES								
	0	1	2	3	4	5	6	7	8
325		1							
350			1						
375				1	1				
400				1	2				
425			3	3	4	2			
450	1	10	29	15	5	2			
475	1	14	29	15	11	7	1	1	
500	2	3	7	6	4	1			

TABLE 16

*To compare children from low grade parents taken from mass cultures and raised in pairs, with children from high grade parents that have come from continuously inbred lines, selected for increase in bristle numbers*

BOTTLE NO.	GRADE OF PAR- ENTS	OFFSPRING—NOS. EXTRA BRISTLES									MEANS
		0	1	2	3	4	5	6	7	8	
<i>Parents from Mass cultures</i>											
554	2:2	1	15	36	32	25	18	3			3.00
557	2:2	3	15	26	12	16	9	3	2		2.84
561	1:2	1	1	3	5	5	3	1	1	1	4.00
<i>Parents from 16th generation of inbreeding and selecting up</i>											
555	5:5		1	11	26	14	4	4	1		3.55
556	5:5	4	32	61	45	24	9				2.45
559	5:5	6	33	39	42	31	17	6		1	2.80

than the inbred flies mated in pairs. It is also found that the first flies that hatch from a new culture bottle are larger and have increased bristle numbers and low grade pairs separated from mass cultures give children of as high grades as are found in the inbred lines raised at the same time (table 16). The mass cultures, from which the parents in this table were taken, were started from the 5th and 6th selected generations and had been running eight months. The three inbred families shown from the 16th generation are the only ones mated very nearly at the same time as the three pairs taken from the mass cultures. This table has a deep significance in relation to the accomplishments of selection in the ten generations between the 6th to 16th.

## 2. DISCUSSION

Thus there is evidence to show that flies below a certain size are apt to have fewer extra bristles than larger flies and that the size is largely dependent upon the condition and amount of food, or, more generally, on the environment; further, that males are shorter and have fewer extra bristles than females, and that the differences between mass cultures and the inbred lines disappear when the flies from the mass cultures are bred in pairs. It seems as though the small underfed flies do not have enough material to develop as many extra bristles as the larger flies can.

The measurements used in the correlation tables do not make very satisfactory data, as the distention of the abdomen and so, the length of the fly, varies with the amount of food contained, but, however little light the tables may give as to the actual amount of correlation and influence of the environment, it is believed that the errors in measurement due to the varying abdominal contents are not great enough to prevent the conclusion that such an influence of environment does exist. The fact of most importance at present, whatever its explanation may be, is that the numbers of extra bristles are influenced in some direct or indirect way by the conditions in the bottles.

With these facts in mind it will be well to return to the generally low percentage of extras in the  $F_2$  of crosses with normals of other races. It has previously been shown that apparent normals may none-the-less be homozygous for extra bristles; a plausible explanation of this fact seems to be the influence of food.

### 3. TEMPERATURE

The regularity with which a new bottle produces an increase in bristle numbers, if it follows a falling off at the end of the preceding bottle, shows that these variations are not mainly due to temperature. This does not say that temperature may not have a real, however slight, influence on bristle development.

A series of preliminary experiments were performed to test the influence of temperature. Sister matings were raised in various temperatures, constant to a small fraction of a degree contigrade. In one experiment different sets of eggs from the same parents were raised in various temperatures and compared with controls in room temperatures. Two main questions were to be answered: Can the number of extra bristles be increased by using higher or lower temperatures? Do constant temperatures, irrespective of the degrees, influence the variability of bristles and thus indicate an influence of temperature? In the nine matings employed, 1860 flies were observed. The data were all arranged in curves, males and females separated and together, but no clear evidence was found to show that temperature had any influence, either in producing higher or lower numbers of extra bristles or by changing their variability. It is very clear that temperature influences the bacterial and fungal floriae of the fermenting banana, and due to the chemical activity of the food, the environment of the developing flies goes through wide variations, as is witnessed by the multitude of different shades of odors from a single bottle during its productivity. Should any relation between temperature and bristle number be found in later investigations, it would be extremely difficult to show that this supposed relation was not an indirect effect, act-

ing through the influence on the food. There can be no question that extra bristles are not influenced by temperature in the way that Miss M. Hoge found applied to extra legs.

#### DISCUSSION

The question in relation to which these experiments have most interest is whether Mendelian units can be modified by selection, and whether selection can accomplish anything more than a sifting and sorting of hereditary elements whose origin is still unknown. The tendency is either to hold that the hereditary elements can never be modified by simply propagating certain ones, or to hold that there is no integrity of such seeming elements or factors, and that almost anything can be accomplished by sufficiently long and painstaking selection.

Some of the observations will admit both interpretations. According to the selectionist's view, the modification of the extra bristles appearing in a cross with normal, indicates that a factor has been modified. The restricting factor of the normal gamete may have transferred some of its restrictive properties to its allelomorphic mate in the *extra* germplasm, so that all the children formed by the union of two so modified *extra* gametes, would have fewer extra bristles than if the gametes had been unmodified. Selection made steady advances at first, showing that the gametes of flies of higher grades must be somewhat different from those of the lower grades, and therefore, the factor for extra bristles must be a variable thing, however truly a Mendelian unit. That the progress of selection does not continue after the sixth generation may only mean that the conditions have been so irregular or unfavorable that the real phenomena of the germplasm were entirely veiled.

On the other hand, these same facts may be used to form the basis of an interpretation involving one or more smaller, or accessory, restricting factors, which are found in many low bristled flies (some flies may lack all restricting factors, and still be low grade on account of their small size). The more and

more complete removal of these in successive generations by selection will permit the numbers of bristles to gradually increase. However, when all the flies become homozygous for the absence of the accessory restricting factors, no further increase by selection could be expected. Since there were no greater irregularities in the environment apparent after, than before, the sixth generation, and since flies from the fifth, sixth and seventh generations were being raised on the same lot of food and at the same time, the other interpretation of the failure of selection becomes very weak. And when the evidence showing that there is apparently no genetic difference in the later generations between flies of different extra bristle grades, is reviewed, including as it does the weighty finding that seemingly normal parents may produce higher grade *extra* children than preceding high selected ancestors and the conclusion that after nine selections, high and low bristle grades give the same result in crosses, when all these facts are born in mind, it will be realized that the interpretation of the results by accessory factors seems in closer agreement with the facts than does the alternative hypothesis.

The hypothesis of accessory factors needs no elaboration or change to explain the phenomena that accompany crosses with a normal race. The main factor for restriction keeps the number of bristles down to four whenever it is present, so the accessory factors can only be detected when the main one is absent. For this reason a simple Mendelian ratio may be found. From the selected flies some if not all the accessory factors have been removed. These would be present in the normal race used in the cross, and, due to the segregation of these independent restrictors, one would find in  $F_2$  among the flies lacking the main restrictor, all combinations of the accessory factors, forming groups with various restrictive powers from strong to weak. The strongly restrictive groups would make the bristle numbers lower than in the uncrossed flies under the same conditions, while the weakly restrictive groups would make slight or no modification. This would result in an increase in the proportions of flies with few extra bristles, yet the high bristle grades would still be found. This is shown to be the case in figure 2,

where the inbred and extracted distributions are compared. The first set of curves show this fairly well, but in the second set, in which the extra parents had been selected much longer, the phenomenon is very clear. On the other hypothesis, if the factor for extra bristles were modified in  $F_1$ , one would expect to find the whole curve of the  $F_2$  *extras* lowered, and another supposition would have to be made to explain the occurrence of high grades. Such an added supposition would involve the modification of the factor, sometimes in various degrees, and other times not at all—a supposition easy to make (Castle '14) but difficult to explain.

That there is a greater modification in the  $F_2$  extracted *extras* when the *extra* parents had been selected for several generations, than when they had not been selected, is the result expected if the selection had accomplished no more than to drop out certain accessory restricting factors, which being present in the wild parent would produce their effect in the second generation at the normal end of the distribution just as strongly as they did in the crosses before selection. To interpret this without using accessory factors one would have to suppose that a factor for extra bristles, that had been made more extra by selection, was more susceptible to contamination, just as squeezing a sponge will make it take up more water; but as has been shown, this kind of a mechanism will not explain the occurrence of the high extremes in this modified distribution. When one considers that the extra bristled condition is due, not to a single factor of which anything is known, but to the nucleus of determiners that carry the main heritage, such a modification hypothesis becomes vague and confused.

The occurrence of *extras* in the  $F_1$  of the crosses may be explained by incomplete dominance, in which case the proportion of  $F_2$  extracted *extras* should be too high instead of too low. Their occurrence may be explained by a heterozygous condition of the restricting factors in the wild New York 1912 race, which had been escaped being weeded out by selection. The occurrence of *extras* in the New York 1912 race suggests such a heterozygous condition. It seems probable that the slightly low propor-

tions of *extras* in  $F_2$  is connected with the modification of their distribution. A strongly restrictive group of accessory restricting factors, even in the absence of the main restrictor may produce a normal fly, especially if the fly happens to be small.

Much of the recent genetics work supports the principle of accessory or multiple factors. According to this principle more than one independent factor may influence a single character. Besides the cases which prove unquestionably the existence of such multiple factors by means of definite ratios, as the ligula in oats and the red grain in wheat (Nilsson-Ehle '09), yellow endosperm in maize, (East and Hayes, '11) and the triangular form of capsule in shepherds purse (Shull '14) there are a large number of investigations in which the presence of multiple factors is strongly indicated. Since the author has already discussed such cases (MacDowell '14 a and b) it will be necessary only to add references to the recent work of Wichler ('13), Ikeno ('14), Hayes ('14), Davenport ('13), Lotsy ('13), Phillips ('14) and Punnett ('14), and to call attention to the critical discussion of Shull ('14). Investigations showing transgressing segregation, in which the  $F_2$  distributions form continuous gradations but with modes dividing the individuals into groups corresponding to 3 : 1, or 1 : 2 : 1, (Balls '07, Leake '11, Biffen '05) have been interpreted by an hypothesis involving one main factor and accessory factors, similar to the one employed above. Still closer resemblance to the work herein described is that of Castle and Phillips ('14) with piebald rats. They dealt with a variable character that proved to be influenced by a Mendelian factor. The distribution of the variations of this character was modified by crosses. No hypothesis other than that of accessory factors could be found to explain all the results. However, Castle still holds that the continued success of selection goes to prove the modifiability of a Mendelian factor. The main difference between the investigations on the piebald rats and the extra bristled flies, aside from the fact that no minus race of flies has been established, lies in the fact that in the case of the flies the progress of selection does not seem to continue after the sixth generation, while in the rats it appears to continue as far as the

selections have been made. Another difference that becomes illuminating in the light of these facts is that the flies have been inbred absolutely, while in the rats the inbreeding was of a fairly low degree. Return selection proved equally effective in the piebald rats. If the interpretation of accessory factors be accepted for the results of crossing the rats, it is evident that at least part of the success of selection was due to the sorting out of these accessory factors. Now the fact of a successful return selection can not be sighted in this case to prove the theory that a factor has been modified, since as long as selection was showing steady progress, it should be possible to start a successful return selection. As long as the original selection was progressing there still is evidence of some heterozygosis of the accessory factors. This would afford a basis for the return selection. At this time any positive statement would be premature, but the results given by an attempted return selection of the extra bristles after the upward progress had seemed to stop, appear to indicate that this return selection is ineffective. Finally the more complicated hypothesis of Pearl ('12) supported by his extensive investigations on fecundity in fowls, must be sighted as one based fundamentally on the conception of multiple factors.

Taken then on their own merits, the results presented in this paper do not give critical evidence in support of either the hypothesis of modification or of accessory factors. However, the failure of selection after success for six generations and the probable genetic equality of the various bristle grades in the later generations, seem to bear the balance strongly towards the hypothesis of accessory factors. Taken in the light of much of the recent genetics investigations, many of which have close theoretical similarities, it is almost impossible to avoid the conclusion that the interpretation of accessory factors is the more probable. Besides, this hypothesis affords a more thinkable mechanism and is more readily understood and tested. For these reasons this interpretation has been adopted at least as a working hypothesis upon which to base further investigations. Experiments are already under way to attempt the isolation of accessory factors and, by crossing, to prove unquestionably their existence.

## CONCLUSIONS

From a pair of wild flies a race of *Drosophila ampelophila* has been established which has regularly more than the normal four thoracic bristles.

By selecting high grade parents and inbreeding brother to sister, the number of extra bristles was gradually increased for six generations.

From the seventh to the eleventh generations fluctuations were found showing no further increase.

The maintenance of the high grades of extra bristles does not depend upon selection as, low grade parents from mass cultures started from the fifth and sixth generations that have run eight months, when raised in single pairs, give as high grade offspring as inbred and selected parents mated at the same time.

A Mendelian factor is involved in the inheritance of extra bristles, and as *normal* dominates *extra*, this may be regarded as a dominant factor that restricts the number of bristles to four.

This factor is not sex linked, although males are apt to have fewer extra bristles than females.

The extracted extra bristled flies have a lower distribution than that of the inbred flies of the corresponding generation, although the high extremes of the inbred race are also found among the extracted *extras*.

There is a greater difference between the inbred and extracted distributions when the cross is made after eight selections than when made after only one selection.

Environment influences the number of extra bristles, and since small flies are not apt to have as many extra bristles as large ones, it appears that the amount of food eaten is an important factor.

From the above statement an explanation may be found for the fact that an apparently normal fly may be genetically homozygous for extra bristles.

The hypothesis of accessory factors will explain all the facts, and that of modification of a Mendelian factor may be employed to interpret most of them.

The similarity with much recent work that has given more or less positive evidence of multiple and duplicate factors, persuades the author that the hypothesis of accessory factors is probably the best one for the facts.

The following hypothesis is adopted upon which to base further work: the extra bristles in *Drosophila ampelophila* are due to the absence of one main restricting factor, and their number is also influenced by accessory restricting factors, which, in the absence of the main one, produce flies with reduced numbers of extra bristles.

October, 1914.

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# CHANGES IN THE RELATIVE WEIGHTS OF THE VARIOUS PARTS, SYSTEMS AND ORGANS OF YOUNG ALBINO RATS HELD AT CONSTANT BODY-WEIGHT BY UNDERFEEDING FOR VARIOUS PERIODS

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When young, actively growing animals are held at constant body-weight by underfeeding for considerable periods, one of three results might be expected *a priori* concerning the weights of the various organs and parts. (1) Since the body-weight

remains constant, the weights of the individual organs and parts might also remain constant. (2) Since the normal growth rates are known to vary in the different organs and parts, one might expect those organs with the strongest normal growth tendency to increase in weight at the expense of the remainder of the body. (3) Since, however, the food-supply available is insufficient for both maintenance and growth, the animals are actually in a condition of chronic inanition; and changes might be expected to occur corresponding to those which have been observed in adults during inanition.

A few observations have been recorded upon changes in young animals in certain organs under these conditions, notably by Waters ('08), Aron ('11 and '14) and Donaldson ('11). A more extended and complete analysis of the changes in the organism under these conditions seemed highly desirable, and therefore the present investigation was undertaken. The work was begun at the University of Missouri, and continued at the University of Minnesota with the aid of a special grant from the research fund of the Graduate School. This grant was used to employ a research assistant, who cared for the animals and assisted in the dissections, weighings, calculations, etc. An abstract of the present paper has been published (Jackson '15 b).

#### MATERIAL AND METHODS

The albino rat (*Mus norvegicus albinus*) was chosen for use in this experiment. It is a convenient animal on account of its comparatively small size, rapid growth and hardiness under experimental conditions. It is also practically the only mammal whose growth norm (including variability) for the whole body and for the various parts, systems and organs throughout the post-natal life cycle is even approximately known. The effects of inanition upon the adult rat have also been worked out (Jackson '15 a, '15 c) and are valuable for comparison.

The material used in the present experiment included ten litters (and one individual from an eleventh litter) as shown in table 1.

TABLE I  
*Litters used in experiments*

LITTER NO.	SOURCE	NO. AND SEX OF EACH LITTER	NO. TEST ANIMALS	CONSTANT WEIGHT PERIOD	NO. CONTROL ANIMALS	KILLED AT AGE OF
L 4...	Missouri	{ 1 M. 4 F.	{ 1 M. 3 F.	3-6 wks.	1 F.	6 wks.
L 2...		{ 3 M. 2 F.	{ 1 M. 1 F.	6-32 wks.	{ 2 M. 1 F.	32 wks.
L 3...		{ 4 M. 2 F.	{ 2 M. 1 F.	10-35 wks.	{ 1 M. 2 F.	35 wks.
S 5...	Minneapolis	{ 3 M. 8 F.	{ 1 M. 1 F. 5 F.	{ 3-8 wks. 3-10 wks.	{ 1 M. 1 F. 1 M. 1 F.	{ 3 wks. 10 wks.
S 6...		{ 2 M. 4 F.	{ 1 M. 3 F.	3-10 wks.	{ 1 M. 1 F.	10 wks.
S 7...		{ 4 M. 2 F.	{ 3 M. 1 F.	3-10 wks.	{ 1 M. 1 F.	3 wks.
S 11...		{ 2 M. 4 F.	{ 1 M. 2 F.	3-10 wks.	{ 1 M. 1 F. 1 F.	{ 3 wks. 10 wks.
S 12...		{ 5 M. 3 F.	{ 3 M. 3 F.	3-10 wks.	{ 1 M. 1 M.	{ 3 wks. 10 wks.
S 10...		{ 1 M. 2 F.	{ 1 F.	3-16 wks.	{ 1 M. 1 F.	3 wks.
28....		{ 4 M. 4 F.	{ 2 M. 2 F. 1 F.	{ 3-6 wks. 3-8 wks.	{ 1 M. 1 F.	{ 3 wks. 6 wks.
22....		1 F.	1 F.	3-13 wks.		
Total		29 M. 36 F.	15 M. 25 F.		13 M. 12 F.	

As noted in this table, three of the litters are from the rat colony at the University of Missouri, and the remainder from a local colony at the University of Minnesota. There are included twenty-nine males and thirty-six females, a total of sixty-five. In addition, two rats not listed in tables 1 and 2 were used as additional controls at three weeks in the study of the skeleton (table 7).

In most cases, the experiment began when the rats were three weeks of age (time of weaning). These rats were held at con-

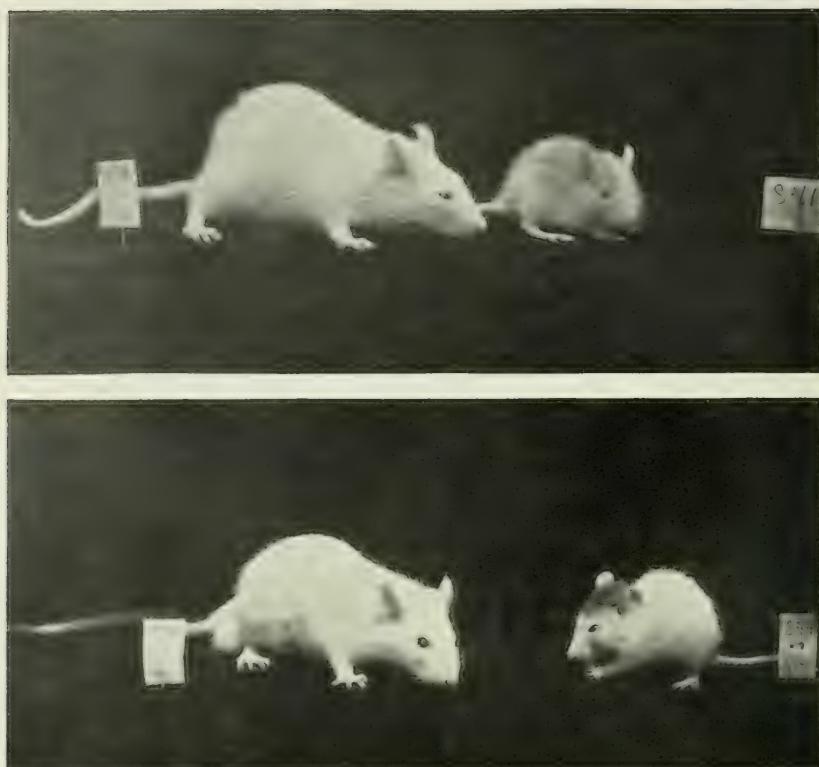


Fig. 1 Photograph showing four albino rats at the age of ten weeks. The larger rats represent the normal, full-fed controls. The smaller rats are from the same litter, but have been held by underfeeding at constant body-weight since the age of three weeks. The rats are pure albinos (*Mus norvegicus albinus*), the dark spot on the head of one being an artificial mark of identification.

stant body-weight for varying periods, from the age of three weeks up to the age of six weeks (8 rats) to eight weeks (3), to ten weeks (22), to thirteen weeks (1) and to sixteen weeks (1). A few were held at constant body-weight beginning at later periods,—from age of six weeks to age of thirty-two weeks (2), and from age of ten weeks to age of thirty-six weeks (3). Controls (25 in all) were also killed at the beginning and at the end of these time periods. At ten weeks of age, the normal controls are half-grown rats, sexually mature, of nearly adult proportions.

and five or six times as heavy as those held at constant body-weight from the age of three weeks (fig. 1). In addition, a large number of observations upon the normal rat previously published (by Donaldson, Hatai, Jackson, Lowrey and others) are available for comparison. Of the animals used in this experiment, the distribution of sexes is given in table 1.

In table 2, the net body-weight (gross body-weight, including intestinal contents, is slightly higher), of the animals used is indicated. At each time period the average weight (and range) for each sex is given, for both controls and test animals held at constant body-weight. The cards containing the original individual records for all animals used will be deposited in The Wistar Institute of Anatomy, where they may be consulted if desired.

The rats were kept in ordinary wire cages (with wire-net bottoms, allowing the feces to drop through) and were individually weighed daily before feeding. Those under experiment

TABLE 2

*Net body-weight of rats when killed; average weight and range indicated*

CONTROLS (FULL-FED)			EXPERIMENTED (BODY-WEIGHT HELD CONSTANT)		
Controls killed at	No. and sex	Weight and range: grams	Weight and range: grams	No. and sex	Test animals constant for
3 weeks..	{ 6 M.	24.4 (19.0-32.6)			
	{ 5 F.	24.7 (21.1-30.4)			
6 weeks..	{ 1 M.	42.3	22.4 (20.5-23.6)	3 M. {	3-6 weeks
	{ 1 F.	42.4	22.0 (20.6-23.8)	5 F. {	
8 weeks			18.1	1 M. {	3-8 weeks
			21.2 (17.8-24.6)	2 F. {	
10 weeks	{ 3 M.	155 (141-167)	24.7 (20.7-31.4)	8 M. {	3-10 weeks
	{ 3 F.	114 (109-118)	23.3 (18.5-31.0)	14 F. {	
32 weeks..	{ 2 M.		25.5	1 F. {	3-13 weeks
	{ 1 F.	209 (203-215)	26.0	1 F. {	
35 weeks..	{ 1 M.	166	43.7	1 M. {	3-16 weeks
	{ 2 F.	238	50.4	1 F. {	
		158 (153-162)	79.6 (73.6-85.5)	2 M. {	6-32 weeks
			74.2	1 F. {	
Total	13 M.			15 M.	
	12 F.			25 F.	

were fed an amount just sufficient to hold them constant at the initial body-weight. Of course slight fluctuations in the gross weight were unavoidable, but they rarely exceeded one gram above or below the initial weight. The food used was in all cases whole wheat (Graham) bread soaked in whole milk. Previous experience has shown that, at least up to the age of one year, albino rats thrive and develop normally upon this simple diet. Water *ad libitum* was also supplied.

It is a curious fact that under these circumstances the amount of food necessary for maintenance of body-weight apparently decreases as the experiment proceeds. Thus rats of about 25 grams gross body-weight when three weeks of age at the beginning of the experiment will at ordinary room temperature require about 5 grams of milk-soaked bread daily for maintenance. Later, this will usually decrease to an average of about 3 grams toward the age of ten weeks.<sup>1</sup> This is the opposite of what might be expected: (1) because at later periods the amount of available food supply stored in the body has been greatly diminished; and (2) because the animals held at constant body-weight almost invariably become much more active, requiring a greater expenditure of energy. Possibly the smaller amount of food required to maintain the animals at the later periods may be due to a greater absorption of water, thus maintaining a body-weight which would otherwise decline with the given amount of food. It is well known that during inanition in general the

<sup>1</sup> Two examples may be cited. Six rats of litter No. 12 were held at constant body-weight (within a range of 1 gram) from the age of three weeks on June 21, 1914, average body-weight 23.6 grams, for seven weeks to the age of ten weeks on August 6, 1914, at which time the average gross body-weight was 23.8 grams. The average daily food-supply of whole wheat (Graham) bread soaked in whole milk for the seven consecutive weeks of the experiment was as follows: 5.1, 3.9, 3.7, 3.5, 3.3, 2.7, 2.7 grams. Similarly, six rats of litter No. 13, average weight 23.1 grams at three weeks of age on June 28, 1914, were held at constant weight for seven weeks until August 12, 1914, when at ten weeks of age their average gross weight was 22.6 grams. Their average daily food-supply for the seven consecutive weeks was as follows: 5.3, 5.0, 4.1, 3.9, 3.3, 3.2, 2.9 grams. In all cases water (city supply, from the Mississippi river) was supplied *ad libitum*. The diminishing amount of food necessary for maintenance cannot be explained as due to increasing temperature, as this was fairly constant. Moreover, a similar condition has been found in other litters at all seasons of the year.

percentage of water-content of the body increases, and it is quite probable that during chronic inanition resulting from maintenance of a young, growing animal at constant body-weight the amount of living protoplasm in the body decreases. If the amount of metabolism is thereby decreased, a smaller food-supply would suffice for maintenance.

As will be shown later, on account of the intensity of the growth-inpulse, especially during the earlier periods of inanition, certain growth-changes occur which require the expenditure of energy. It is possible that this energy is supplied by the excess of food above that required for maintenance proper. Another, but less probable, explanation might be that during inanition the food-intake is in some way more economically utilized, a smaller quantity therefore being sufficient for maintenance. In the later stages of inanition, there is probably a decrease in the temperature of the body, which would therefore require less food.

Rats held at constant body-weight from the age of three weeks to ten weeks, while becoming more active as the experiment proceeds, become at the same time less resistant to cold. They may die suddenly if the room temperature is lowered, or even without any apparent cause. Thus up to sixteen weeks, the longest successful period in those beginning at three weeks, it becomes increasingly difficult to maintain them alive at constant body-weight. When the experiment is begun later, the length of the time during which the body-weight can be held constant is considerably increased. Aron ('11) had a similar experience with dogs, finding it necessary after a time to feed sufficiently to increase the initial body-weight somewhat, in order to keep the animals alive. He explains this as due to the gradual exhaustion of available food-substance stored in the various tissues of the body.

At the end of the various age-periods of the experiment, and at the beginning and end for controls, the rats were killed by chloroform and dissected according to the technique described in previous papers (Jackson and Lowrey '12; Jackson '13, '15 c). The parts, systems and organs were carefully weighed, and

portions preserved for microscopic examination (to be considered in a later paper).

As heretofore, in calculating the percentage weights, the *net* body-weight (gross weight less intestinal contents) is taken. The percentage weights of the organs are thus slightly higher than if calculated upon the gross body-weight.

The averages given in the various tables are the arithmetical means of the corresponding individual observations. In view of the comparatively small number of observations and the known variability, especially of some of the organs (cf. Jackson '13), the data are insufficient for treatment by statistical methods, and the values are therefore only fair approximations. They are, however, sufficiently accurate to show some of the more obvious and important changes in the young animal held at constant body-weight. It is hoped that they may be useful as preliminary observations, which may lead to further and more extensive investigations of the various individual organs. In general, the amount of variation found is sufficient to necessitate great caution in drawing conclusions from a small number of observations (sometimes upon a single animal), as frequently happens in experimental work.

#### LENGTHS OF BODY AND TAIL

The body-length is measured from the tip of the nose to the anus, and the tail-length from the anus to the tip of the tail. The measurements were taken immediately after death, the body and tail being extended by very slight tension. Measurements during life are not practicable, although they might be obtained by the use of anesthetics.

In order therefore to discern the changes in the lengths of body and tail while the body-weight is held constant, it was necessary first to determine these measurements on the normal animal. For this purpose, 450 observations (267 males, 183 females) were available, varying from newborn to about 400 grams body-weight. Of these, 277 (130 males, 147 females) were from the Missouri rats described in a previous paper (Jackson '13), and 25 (13 males, 12 females) from Minnesota. For

the remaining 148 (124 males, 24 females) observations upon rats from the colony at The Wistar Institute in Philadelphia, I am indebted to Professor Donaldson and Dr. Hatai. A careful examination revealed no essential differences in the relations of body and tail-lengths according to the source of the rats, so they were all combined into a single series.

The general relations of body and tail-lengths are evident from table 3 (not including the Wistar data, in which the age was usually unknown).

The average ratio of tail to body-length in table 3 was obtained by calculating the ratio for each individual separately, and then taking the mean of the individual ratios. The results are thereby somewhat more accurate than would be obtained by simply taking the ratios of the *average* tail and body-lengths, though the difference is not great.

TABLE 3  
*Changes in lengths of body and tail*

AGE	NUMBER OF OBSERVATIONS		AVERAGE NET BODY-WEIGHT: GRAMS		AVERAGE BODY-LENGTH: MM.		AVERAGE TAIL-LENGTH: MM.		AVERAGE RATIO OF TAIL TO BODY-LENGTH		
	male	female	male	female	male	female	male	female	male	female	total

*a. Normal rats (full-fed)*

Newborn .....	32	36	4.8	4.6	48.7	45.5	16.9	17.5	0.35	0.37	0.36
1 week.....	23	27	9.6	9.7	64.5	63.3	29.4	31.4	0.46	0.50	0.48
3 weeks.....	22	22	21.7	18.7	90.4	86.1	59.1	57.9	0.65	0.67	0.66
6 weeks.....	21	16	47.4	45.4	128.0	124.0	110.0	110.0	0.86	0.90	0.88
10 weeks.....	19	15	128.0	99.4	173.0	160.0	150.0	143.0	0.87	0.89	0.88
5 to 13 mo....	16	34	177.7	148.0	190.0	183.0	162.0	168.0	0.86	0.90	0.88

*b. Rats under experiment (body-weight held constant)*

(from age of)											
3 to 6 weeks...	3	4	22.4	22.1	98.0	94.4	72.3	77.4	0.74	0.82	0.78
3 to 8 weeks...	1	2	18.1	21.2	94.5	92.0	78.0	80.0	0.83	0.87	0.84
3 to 10 weeks.	8	14	24.7	23.3	105.2	99.6	86.2	83.9	0.82	0.85	0.84
3 to 13 weeks.	0	1		25.5		91.0		85.0		0.93	0.93
3 to 16 weeks.	0	1		26.0		90.0		95.0		1.06	1.06
6 to 32 weeks.	1	1	43.7	50.5	115.0	115.0	105.0	105.0	0.91	0.91	0.91
10 to 35 weeks	2	1	79.6	74.2	140.0	135.0	117.0	115.0	0.84	0.85	0.85

Two facts concerning the relative length of the tail in the normal albino rat are apparent from table 3 a. In the first place, it is evident that at all ages the ratio of the tail-length to the body-length is slightly greater in the female than in the male; that is, the female is relatively long-tailed. By a comparison of the ratios, it appears that the tail of the female on the average is about 4 or 5 per cent longer.

In the second place, it appears that the ratio of tail-length to body-length increases in the normal rat from an average of about 0.36 at birth to 0.48 at one week, 0.66 at three weeks, and 0.88 from the age of six weeks upward. That is, the tail becomes progressively relatively longer, being relatively more than twice as long in the adult as at birth. The acceleration of the tail-growth at such a later period may be cited as an instance of the general law of cranio-caudal progression in development (Jackson '09).

Turning now to the tail-ratio in the rats held at constant body-weight, as shown in table 3 b, and also indicated in the chart in figure 2, it is clear that in rats beginning at three weeks of age there has been a very decided increase in the tail-ratio. The tail at this age evidently continues to elongate, even though the body-weight has been held constant, so that the tail-ratio approaches (although it does not usually quite reach) the normal ratio for rats of corresponding age under normal conditions of growth.

If we compare the absolute lengths of tail and body in the normal rat at three weeks (table 3 a) with those in rats held at constant body-weight from the age of three weeks to the age of six, eight, ten, thirteen and sixteen weeks, it appears that there has also been a slight increase in the absolute length of the body. The increase in tail-length is considerably greater, however, so the tail-ratio increases as above stated.

The larger number of rats were held constant from the age of three to the age of ten weeks. Since the average body-weight for the normal series at three weeks is slightly lower than that of the series held constant to ten weeks of age, I have obtained a new normal series of higher body-weight for direct comparison

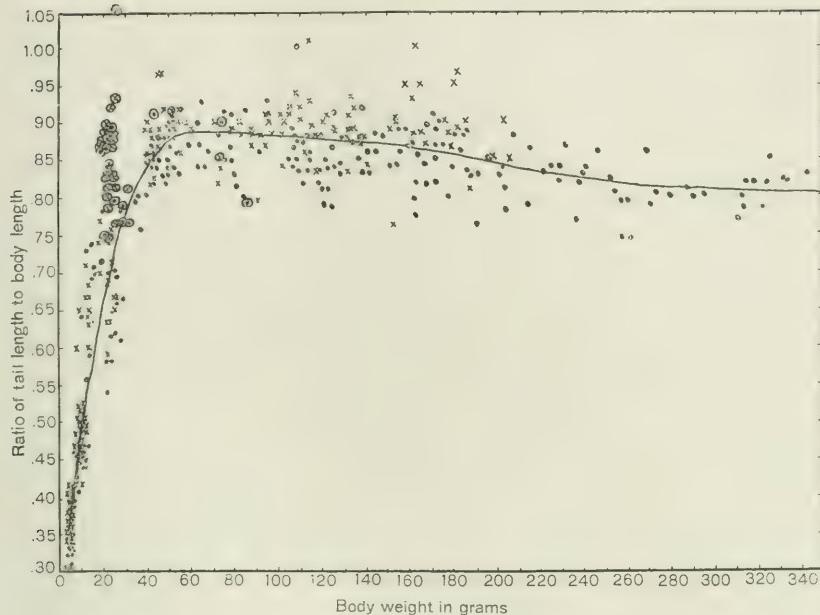


Fig. 2 Chart showing the change in the ratio of tail-length to body (nose-anus) length in the albino rat from birth to maturity. Males are indicated by rounded dots, females by  $\times$ . The individuals held at constant body-weight during the experiment are encircled. The curve is drawn through points representing the means for the normal individuals at various periods, sexes combined.

(by dropping out the lighter rats of the series given in table 3 a). Thus in sixteen normal males aged three weeks, average net body-weight is 24.2 grams, the average body-length is 94.5 mm. and tail-length is 61.6 mm. (compared with 105.2 mm. body-length and 86.2 mm. tail-length in the 3 to 10 weeks experiment). Similarly, in eight normal females at three weeks, average net body-weight 23.2 grams, the average body-length is 91.1 mm., and tail-length is 63.4 mm. (compared with 99.6 mm. body-length and 83.9 mm. tail-length in the 3 to 10 weeks experiment). In other words, while the body has increased about one-tenth in length, the tail has increased over one-third, the body-weight being held constant from the age of three to ten weeks.

In order to show more clearly this change in the ratio of tail-length to body-length, the individual ratios corresponding to the 450 observations on rats from all sources (including the 148 Wistar rats) were plotted according to body-weight in figure 2. As the sexes are distinguished in the entries, it is evident that the females tend to have a higher tail-ratio. The curve has been drawn through the averages at various periods (sexes combined). It is smoothed free hand, as the labor of constructing the curve more accurately by mathematical methods did not seem justified.

Special attention is called to one apparent discrepancy between the curve in figure 2 and the data in table 3 a. In the latter, it appears that the tail reaches at six weeks an apparent maximum ratio of 0.88 (sexes combined), which is maintained nearly constant at succeeding periods. In the chart, however, it is seen that in rats above 300 grams the average ratio drops to nearly 0.80. These heavy rats are nearly all from the Wistar series, and are all males. It is therefore evident that the drop in the tail-ratio curve is in part due to the fact that no females are included in the higher body-weights. Even taking this into account, however, there is still a decrease in the tail-ratio of the male from a maximum of about 0.86 or 0.87 in rats between 50 and 200 grams body-weight, to nearly 0.80 in rats above 250 grams. (In 7 male rats between 350 and 400 grams,<sup>\*</sup>not shown in figure 2, the ratios were slightly above the 0.80 line, the average being 0.81).<sup>2</sup>

<sup>2</sup> Since the completion of the present paper, I have received, through the courtesy of Professor Donaldson, a manuscript copy of reference tables compiled at The Wistar Institute by formulas for various measurements of the albino rat. These include the body-lengths and tail-lengths, by sexes, from newborn to adult. From these data I have calculated the tail-ratios and find the result in general agreement with the curve shown in figure 2. The tail-ratios calculated from the Wistar tables are somewhat lower, corresponding to body-weights from 30 to 100 grams, however. They also increase steadily, so that at body-weights above 200 grams they lie slightly above the curve in figure 2. The tail-ratio according to the Wistar tables is about 4 per cent higher in the female throughout, when the sexes of equal body-length or body-weight are compared. In rats above 300 grams body-weight, the tail-ratio is about 0.86 in the male and 0.89 in the female.

The two observations on rats held constant from the age of three weeks to the ages of thirteen and sixteen weeks would seem to indicate a continuation of the process of elongation of the tail, even beyond the normal ratio at corresponding age, but the number of observations is too small for definite conclusion.

The number of animals beginning the experiment at later ages, body-weight constant from age of six to age of thirty-two weeks (2) and from ten to thirty-five weeks (3), is also too small to draw any very positive conclusions. However, so far as they go, they indicate (table 3 b; fig. 2) that *between the ages of six and thirty-five weeks there is no marked change in the tail-ratio of rats held at constant body-weight.*

It will be observed that also in the normal rats between six and thirty-five weeks of age there is no apparent change in the tail-ratio, whereas between three and six weeks of age there is normally a decided increase in the tail-ratio. The lengths of the body and tail are of course determined primarily by the growth of the skeleton. I would therefore interpret the results concerning the lengths of tail and body as follows. In young, growing rats held at constant body-weight, the body and tail tend to increase so as to assume the normal ratio at corresponding ages. This is due to the fact that, as will appear later, the skeleton continues to grow in a normal manner (though at a reduced rate) in animals held at constant body-weight.

There is another possible factor in causing the increased tail-ratio in young, growing rats, which may also apply to the similar relative elongation of the tail found in adult rats (cf. Jackson '15 c). Professor Donaldson points out (in a personal communication) that during inanition there may be an arching of the spinal column, producing an actual shortening of the body-length. Such an arching actually does occur, and may be noted especially in young rats during chronic inanition. It is well shown in the stunted rats photographed in figure 1. Of course the greater part of this longitudinal curvature of the spinal column is eliminated by the slight tension exerted in order to straighten out the body when it is measured after death. But it is still

quite possible that this does not entirely eliminate the shortening of the column. In any event, however, this is probably a factor of minor importance in altering the tail-ratio during inanition.

Hatai ('08) in a series of five 'stunted' rats in which growth had been retarded (but not stopped) by a diet of starch-mixtures from age of 30 days up to from 127 to 215 days (the final body-weight being from 70.9 to 113.7 grams) finds the average tail-ratio 0.75 as compared with about 0.82 in controls. He notes that:

The most conspicuous external differences between normal and stunted rats as shown by the stunted rats are in the length of the body and of the tail, both of which were considerably reduced with respect to the body-weight. This peculiar difference, as is seen from the table, holds true in every case. Further, the ratio between the length of the body and that of the tail is considerably less in the stunted rats than in the control rats. . . . Underfeeding therefore produces short tailed individuals.

Recently, however, Dr. Hatai (in a personal communication) states that in other inanition experiments he has obtained different results, and that "rats either grown or kept in a state of chronic inanition (starch feeding, lipoid-free ration and wheat embryo feeding) give a longer tail" in agreement with my results.

Morgulis ('11) in the salamander *Diemyctylus* found a relatively greater shrinkage in the tail than in the body during inanition; while Harms ('09) found the converse to be true in *Triton*.

#### HEAD

The head (table 4; fig. 3) at three weeks normally forms an average of 22.5 per cent of the body, the average net body-weight being 21.2 grams. In the 11 controls at three weeks, the body-weight (24.6 grams) is slightly higher, and the corresponding relative head-weight, 20.6 per cent, somewhat lower. In the rats held constant from the age of three weeks to the ages of six and eight weeks, the average percentage of the head (21.6 per cent and 23.9 per cent) is higher than that of the controls. But the average body-weight in these groups is lower, more

nearly the normal above cited, so it is doubtful whether there is any actual increase in the head-weight (either relative or absolute) during the experiment. The larger ten weeks group, however, is nearly equal to the controls in average body-weight, and shows an apparent increase in head-weight from an average of 5.01 to 5.34 grams, or from 20.6 per cent to 22.7 per cent (table 4; fig. 3). In any event, however, the increase in the head-weight is slight, and is not apparent in the two rats held constant from three to thirteen and sixteen weeks (average of the two is 20.6 per cent). On the other hand, there appears to be a slight increase in the weight of the head in the rats held constant from six to thirty-two weeks (15.2 to 17.7 per cent), and from ten to thirty-five (12 to 14.0 per cent).

On the whole, therefore, the evidence would appear to indicate that in young rats held at constant body-weight for considerable periods of time there is a slight increase in the weight of the head. This is probably due to the increase in skeletal weight, which in the head probably overbalances the decrease in the weight of the

TABLE 4

*The head; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal head at 3 weeks (Jackson '13).....	24	21.2	4.60 (3.44- 5.85)	22.5 (16.3-27.3)
Controls at 3 weeks.....	11	24.5	5.01 (4.70- 5.90)	20.6 (18.3-23.0)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.4	4.83 (4.50- 5.10)	21.6 (19.9-23.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	3	20.2	4.73 (4.60- 4.90)	23.9 (19.9-26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	22	23.8	5.34 (4.60- 6.50)	22.7 (20.4-26.2)
Body-weight constant 10 weeks (age of 3 to 13 weeks)	1	25.5	5.00	19.6
Body-weight constant 13 weeks (age of 3 to 16 weeks)	1	26.0	5.50	21.6
Normal at 6 weeks (Jackson '13).....	42	50.0	7.40 (6.30-9.40)	15.2 (11.3-18.0)
Controls at 6 weeks.....	2	42.4	6.40 (6.20- 6.60)	15.0 (14.6-15.5)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	8.30 (8.20- 8.50)	17.7 (16.8-18.6)
Controls at 10 weeks.....	6	134.0	13.30 (11.10-14.70)	10.1 (8.6-11.7)
Body-weight constant 25 weeks (age of 10 to 35 weeks (normal head for corresponding body-weight of 85 grams forms about 12.0 per cent).....	1	85.5	12.10	14.0
Controls at 32 and 35 weeks.....	6	189.5	20.30 (17.40-26.30)	10.7 (9.8-11.4)

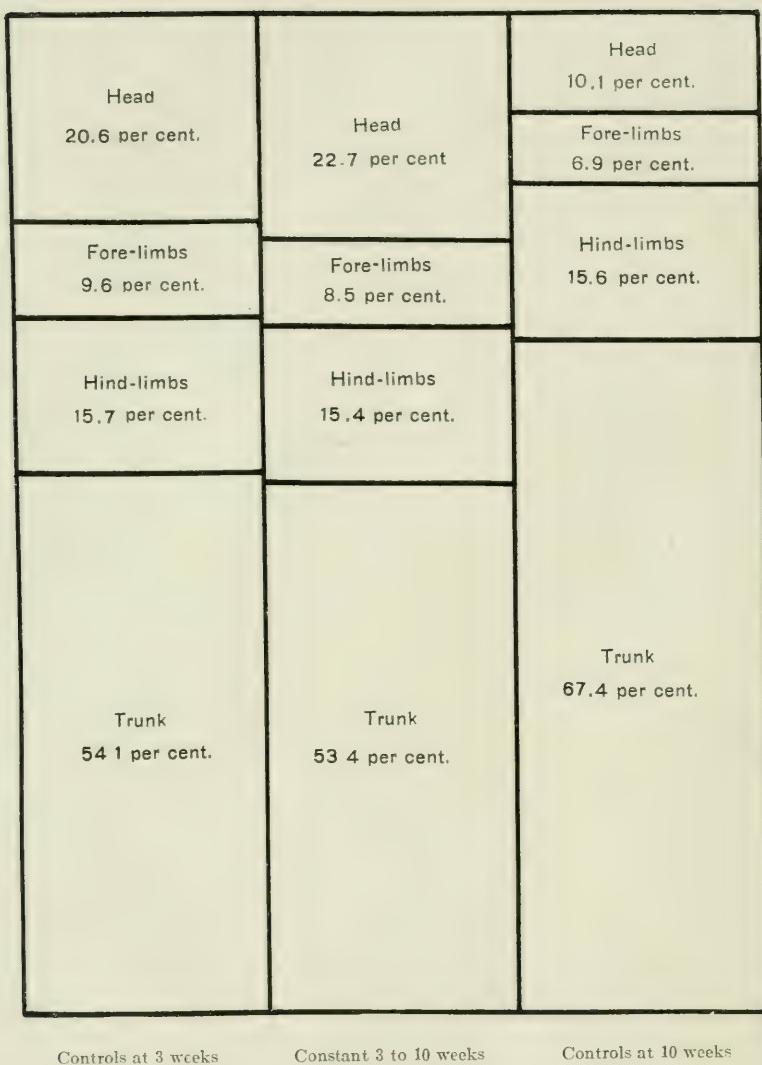


Fig. 3. Diagram representing the average relative (percentage) weights of parts of the body (head, extremities and trunk) in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

integument; although, as will be shown later, the average loss in the integument of the *entire body* is relatively slightly greater than the corresponding skeletal increase (fig. 4).

In both acute and chronic inanition in adult rats (Jackson '15 a, '15 c) the head increases very markedly in relative weight, the loss in absolute weight being but very slight in comparison with the loss in weight of the entire body.

#### EXTREMITIES AND TRUNK

The extremities (table 5; fig. 3) were separated at the shoulder-joint and hip-joint, respectively. There is apparently a slight decrease in the relative weight of the fore-limbs in the young rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks of age. In the case of the rats held constant from three to ten weeks, the apparent decrease is from an average of 9.6 per cent to 8.5 per cent. On account of the small number of observations, however, and the difficulty in separating the limbs (especially the integument) in an absolutely uniform manner, the slight apparent decrease is of doubtful significance.

In the case of the hind-limbs, there is likewise an apparent indication of a slight decrease, but even less marked than in the fore-limbs. The apparent average decrease from 15.7 per cent to 15.4 per cent of the body-weight in the largest group (three to ten weeks), is well within the limits of experimental error.

On the whole, therefore, it is doubtful whether there is any distinct and significant change in the weights of the extremities in young rats held at constant body-weight for considerable periods. A slight loss, however, might be accounted for by the slightly greater loss in the integument (as compared with the gain by the skeleton); especially since the integument of the limbs probably forms a relatively larger part of the limbs than the whole integument does of the whole body.

The trunk was not weighed directly, but its weight was calculated by subtracting from the net body-weight the weight of the head and extremities. From what has been said con-

TABLE 5

*The extremities; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
<i>a. Fore-limbs</i>				
Normal at 3 weeks (Jackson and Lowrey).....	4	25.5	2.36	9.3 (7.4-10.90)
Controls at 3 weeks.....	6	23.8	2.29 (2.00-2.51)	9.6 (8.7-10.60)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	4	23.1	1.68 (1.50-1.90)	7.3 (6.7-8.30)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	1	24.6	1.70	6.9
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	8	22.3	1.84 (1.60-2.20)	8.5 (7.4-9.50)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	1.80	7.1
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	1.80	6.9
Normal at 6 weeks (Jackson and Lowrey).....	4	79.2	5.30	6.7 (5.9-8.22)
Controls at 6 weeks.....	1	42.3	3.00	7.1
Controls at 10 weeks.....	2	127.9	8.90 (7.20-10.60)	6.9 (6.3-7.60)
Normal at 10 weeks (Jackson and Lowrey).....	3	141.9	7.60	5.3 (5.21-5.50)
<i>b. Hind-limbs</i>				
Normal at 3 weeks (Jackson and Lowrey).....	4	25.5	3.80	14.9 (14.3-15.40)
Controls at 3 weeks.....	6	23.8	3.75 (3.20-4.13)	15.7 (14.3-15.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	4	23.1	2.97 (2.55-3.60)	12.8 (11.6-15.10)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	1	24.6	3.40	13.8
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	8	22.3	3.38 (3.00-3.80)	15.4 (14.3-15.90)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	3.20	12.4
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	3.20	12.6
Normal at 6 weeks (Jackson and Lowrey).....	4	79.2	11.80	14.9 (14.6-15.40)
Controls at 6 weeks.....	1	42.3	5.60	13.2
Controls at 10 weeks.....	2	127.9	19.90	15.6 (15.4-15.80)
Normal at 10 weeks (Jackson and Lowrey).....	3	141.9	22.10	15.6 (14.5-16.50)

TABLE 6

*The integument; average absolute weight, average percentage of net body-weight and range indicated*

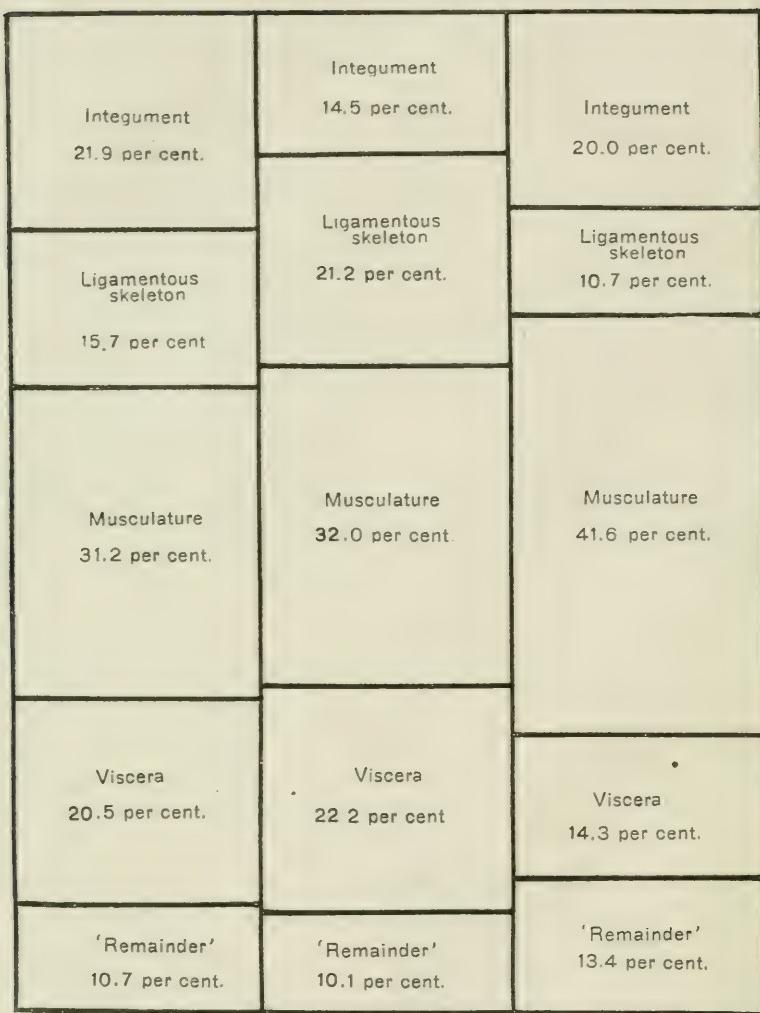
DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal integument at 3 weeks (Jackson and Lowrey).....				
Normal at 3 weeks (Jackson and Lowrey).....	13	24.8	5.55	22.4 (18.7-29.2)
Controls at 3 weeks.....	10	25.1	5.59 (3.86-6.36)	21.9 (18.4-25.2)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	8	22.2	2.81 (2.21-3.21)	12.5 (9.82-14.1)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	3	20.2	2.78 (2.27-3.30)	13.8 (12.6-15.5)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	22	23.8	3.41 (2.63-4.51)	14.5 (12.3-17.5)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	3.50	13.7
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	3.30	12.7
Normal at 6 weeks (Jackson and Lowrey).....	14	64.4	13.46	20.9 (16.7-25.9)
Controls at 6 weeks.....	2	42.4	8.04 (8.00-8.08)	17.0 (15.1-19.0)
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	6.15 (5.70-6.60)	13.0 (12.9-13.1)
Normal at 10 weeks (Jackson and Lowrey).....	10	131.0	24.50	18.7 (15.6-22.3)
Controls at 10 weeks.....	6	134.0	26.70 (19.40-32.10)	20.0 (17.8-22.0)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	14.00 (12.30-17.20)	17.9 (16.6-20.1)
Controls at 32 and 35 weeks.....	6	189.5	38.40 (28.10-55.20)	20.1 (18.0-23.2)

cerning the head and extremities, it follows that there cannot be much change in the trunk-weight, since the probable slight increase in the weight of the head is off-set by a slight decrease in the extremities. In the largest group, however, held at constant body-weight from three to ten weeks of age (fig. 3), the trunk would apparently decrease from an average of 54.1 per cent to 53.4 per cent. This apparent change is so slight as to be (probably) insignificant.

The results concerning the parts of the body therefore fail to indicate any decided change of proportional weights in young animals held for considerable periods at constant body-weight. There is apparently a very small increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to be of doubtful significance. During inanition in adult rats, there is apparently a relative increase in both head and extremities, counterbalanced by a relative decrease in the trunk (Jackson '15 a, '15c).

#### INTEGUMENT

In the rats held at constant body-weight from the age of three weeks to six, eight, ten, thirteen and sixteen weeks, there is a very marked loss in the weight of the integument (including hair and nails; table 6; fig. 4). In the case of the largest (three to ten weeks) group, the decrease is from an average of 21.9 per cent to 14.5 per cent of the body-weight. In terms of absolute weight, the decrease is from 5.30 grams (5.59 grams, less correction on account of difference in body-weight, which averages 25.1 grams at three weeks and 23.8 grams at ten weeks) to 3.41 grams, a decrease of about 36 per cent. The decrease would appear slightly greater if the difference in relative weight of the integument for different initial body-weights were taken into account. There is apparently even greater loss at the other ages. It would appear that this loss (which is perhaps chiefly a loss of fat) occurs rather early, as at six weeks (body-weight held constant three weeks) the loss is as great as at subsequent and longer periods.



Controls at 3 weeks.

Constant 3 to 10 weeks.

Controls at 10 weeks.

Fig. 4 Diagram representing the average relative (percentage) weights of the various systems (integument, skeleton, musculature, viscera and 'remainder') in albino rats held at constant body-weight from the age of three to ten weeks, and in controls at three and at ten weeks of age.

In the rats experimented upon at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) there is also a marked loss in the weight of the skin, though apparently not so great as at the earlier periods.

This loss in the weight of the integument is in striking contrast with the results of inanition in adult rats (Jackson '15 a, '15 c). Here the loss is very nearly proportional to that of the whole body, so the integument nearly maintains its relative (percentage) weight.

From his experiments upon young dogs held at constant body-weight, Aron ('11, p. 29) states that: "The skin shows a slightly higher percentage of the body-weight in those animals kept at a constant weight than in the normal, control dogs. These figures indicate that, while the (body) weight was constant, the skin increased very slightly in weight." The figures cited show the skin in animals held at nearly constant body-weight to form (in four cases) 12.2 to 14.6 per cent of the body-weight, whereas in three corresponding full-fed controls the skin formed 11.2 to 13.0 per cent. Aron, however, overlooks the fact that he is making his comparison with controls at the *end* of the experiment. In order to judge what changes have taken place during the experiment, the comparison must be with normal control animals killed at the *beginning* of the experiment. Aron records but one case which can be used for this purpose. His Dog D (table 13, Experiment IV) killed at the age of 40 days, the beginning of the experiment, with body-weight of 1985 grams shows a skin-weight of 320 grams, or about 16.1 per cent of the body-weight. From Aron's own data, therefore, I would reach the opposite conclusion, viz., that in young dogs held at constant body-weight, the skin suffers a marked loss in weight. This would agree with my results on rats.

#### SKELETON

The skeleton (table 7; fig. 4) was prepared in three ways. The bones, together with the cartilages, periosteum and ligaments, constitute the 'ligamentous skeleton' (table 7 a). The bones and cartilages, after removal of the periosteum and liga-

ments by immersion for about one hour in 1 per cent aqueous 'gold dust' solution at 90°C., constitute the 'cartilaginous skeleton' (table 7 b). Finally the cartilaginous skeleton dried in an oven at 95°C. to constant weight constitutes the 'dry skeleton' (table 7 c).

An examination of the weight of the ligamentous skeleton (table 7 a) reveals the striking fact that while the body-weight

TABLE 7

*The skeleton; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
<i>a. Ligamentous skeleton</i>				
Normal at 3 weeks (Jackson and Lowrey).....	13	24.8	4.12	16.6 (13.1-21.10)
Controls at 3 weeks.....	12	24.5	3.90 (3.100-5.46)	15.7 (13.5-17.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	8	22.2	4.04 (3.140-5.50)	18.0 (14.6-23.90)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	3	20.2	4.74 (4.290-5.50)	23.7 (22.3-24.90)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	22	23.8	4.98 (3.700-7.14)	21.2 (17.7-24.60)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	4.80	18.8
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	5.20	20.0
Normal at 6 weeks (Jackson and Lowrey).....	14	64.4	9.02	14.0 (10.5-20.10)
Controls at 6 weeks.....	2	42.4	5.48 (4.750-6.20)	13.0 (11.2-14.70)
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	7.20 (6.800-7.60)	15.4 (15.1-15.60)
Normal at 10 weeks (Jackson and Lowrey).....	10	131.0	15.30	11.7 (10.0-12.90)
Controls at 10 weeks.....	6	134.0	14.00 (11.400-17.60)	10.7 (8.5-14.10)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	10.30 (9.400-11.60)	13.3 (12.7-13.70)
Controls at 32 and 35 weeks.....	6	189.5	18.60 (15.600-24.30)	9.8 (8.8-11.20)
<i>b. Cartilaginous skeleton (Fresh)</i>				
Controls at 3 weeks.....	6	22.9	2.60 (2.120-3.00)	11.4 (9.0-12.90)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	3	22.9	3.56 (2.930-4.04)	15.5 (13.3-17.00)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	1	24.6	4.50	18.3
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	8	22.4	3.16 (2.420-4.00)	14.6 (11.9-17.10)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	4.00	15.7
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	3.96	15.2
Controls at 10 weeks.....	1	115.0	6.40	5.6
<i>c. Dry skeleton (cartilaginous)</i>				
Controls at 3 weeks.....	5	23.4	0.804 (0.710-0.964)	3.43 (3.17-4.03)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	3	21.9	1.091 (1.033-1.172)	4.98 (4.70-5.13)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	1	24.6	1.351	4.59
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	9	22.2	1.285 (1.076-1.485)	5.84 (4.76-7.00)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	1.068	6.31
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	1.744	6.71
Control at 10 weeks.....	1	115.0	3.420	2.97

is held constant the skeleton continues to increase in weight to a marked degree. In the rats beginning at three weeks, there is an increase in the relative weight of the ligamentous skeleton from 15.7 per cent of the body to 18.0 per cent at six weeks and to an apparent maximum of 23.7 per cent at eight weeks. This latter is probably an exceptional figure, as in the largest group, at ten weeks, the average is 21.2 per cent (fig. 4). This corresponds to an increase from an absolute weight of 3.90 to 4.98 grams, an increase of about 28 per cent (or slightly more, if correction be made for the difference in body-weight, average 24.5 grams at three weeks and 23.8 grams at ten weeks). The two cases carried to thirteen and sixteen weeks, respectively, show a slightly smaller relative increase. The rats used at later and longer periods (ages of six to thirty-two weeks and ten to thirty-five weeks) also show a considerable increase in the skeleton, though relatively less than those beginning at the earlier period.

The data for the cartilaginous skeleton (table 7 b) similarly show a marked increase in rats held at constant body-weight for various periods beginning at three weeks of age. The figures for the largest group (three to ten weeks) indicate an increase from 11.4 per cent to 14.6 per cent of the body. In terms of absolute weight, the increase is from an average of 2.60 grams (body-weight 22.9 grams) to 3.16 grams (body-weight 22.4 grams), an increase of about one-fourth. Subtracting the percentage weights of the cartilaginous skeleton from the corresponding ligamentous skeleton, there is (for the three to ten weeks group) an evident increase of the ligaments and periosteum from 4.3 per cent to 6.6 per cent of the net body-weight. This would indicate that the ligamentous component of the skeleton shares in the marked growth during constant body-weight.

Professor Donaldson (in a personal communication) has kindly supplied a series of observations showing that the cartilaginous skeleton in the normal rat changes from a relative weight of about 10 per cent of the body at 20 grams to 7.5 per cent at 50 grams, 7 per cent at 100 grams and 6.7 per cent in rats above

200 grams. These weights, however, do not include the intervertebral discs.

The data for the dried cartilaginous skeleton (table 7 c) indicate an even greater increase in the dry skeleton of the rats held at constant body-weight. Thus in rats beginning at three weeks the dry substance increases from 3.43 per cent of the body weight to 4.98 per cent at six weeks of age, 5.49 per cent at eight weeks, 5.84 per cent at ten weeks, 6.31 per cent at thirteen weeks and 6.71 per cent at sixteen weeks.

Since the increase in the dry skeleton is relatively greater than that for the (moist) cartilaginous skeleton, it necessarily follows that the skeleton must be losing in percentage of water and gaining in percentage of dry substance. The percentage of dry substance has been calculated for each individual skeleton included in tables 7 a and 7 b, and the averages for each group are as follows: controls at three weeks, 31.4 per cent; constant three to six weeks of age, 33.5 per cent; three to eight weeks, 30.0 per cent; three to ten weeks, 41.7 per cent; three to thirteen weeks, 40.2 per cent; three to sixteen weeks, 44.0 per cent; control at ten weeks, 53.4 per cent.

Lowrey ('13) finds the dry substance of the *ligamentous* skeleton in the normal albino rat to increase from an average of 33.3 per cent at 20 days of age to 39.2 per cent at six weeks, 45.9 per cent at ten weeks, 50.4 per cent at five months and 52.6 per cent at one year.

From the foregoing it is evident that in rats held at constant body-weight beginning at three weeks, the growing cartilaginous skeleton steadily increases its percentage of dry substance. Thus it tends to change the proportions of water and dry substance as during normal growth. The percentage of dry substance does not increase so rapidly with age as during normal growth, however, but lags behind corresponding to the retardation in absolute growth. During inanition in adult rats, on the contrary, there is a relative decrease in the dry substance, and an increase in water-content (Jackson '15 e).

It has already been noted in a previous section ("Lengths of body and tail") that in the rats held at constant body-weight

beginning at the age of three weeks there is an increase in the lengths of both body and tail. The latter increases more rapidly, however, so that it tends to assume the tail-ratio found in normal rats of corresponding age. This indicates that the skeleton not only continues to grow (though at a reduced rate) while the body-weight is held constant, but also tends to *grow in a normal manner*, so as to produce the normal ratio of tail-length and body-length. The preceding paragraphs have shown that the increased growth of skeleton affects the ligamentous as well as the cartilaginous and bony components, and that the chemical composition (percentages of water and dry substance) also changes in a manner tending to assume the normal.

The question naturally arises as to whether the skeletal growth during constant body-weight is merely a growth in mass, or is associated with the normal process of *differentiation*. During the present investigation a few observations have been made upon the development of the normal skeleton, indicating some of the more obvious changes during the age-periods of the rats under experiment, especially between the ages of three and ten weeks. While a detailed study of the developmental changes in the skeleton is reserved for a separate paper, some preliminary conclusions may be noted here.

In skeletons of rats held at constant body-weight from the age of three to the age of ten weeks, the appearance and fusion of certain epiphyses may be noted as in the normal animal during this period, although in most cases the process appears to be retarded somewhat. The following examples may be cited. In the normal skeleton at three weeks of age, the epiphyses at the ends of the vertebral bodies have not appeared; the epiphysis at the lower end of the humerus is well developed, but not fused with the shaft; the maxilla and mandible present each two molars (on each side), with no visible trace of a third. In the normal skeleton at ten weeks, the epiphyses at the ends of the vertebral bodies have appeared, and most of them have united with the corresponding bones; the epiphysis at the lower end of the humerus is firmly fused with the shaft; well developed third molar teeth have appeared, both in the maxilla and in the mandible.

ble. In the skeleton of a rat held at constant body-weight from three to ten weeks of age, most of the epiphyses of the vertebral bodies have appeared, and they usually have united at one end of each bone; the lower epiphysis of the humerus is firmly united with the shaft, as normally at ten weeks; well-developed third molars have appeared, as normally at ten weeks. In a rat held at constant body-weight from age of three to sixteen weeks, the skeletal differentiation was more advanced, corresponding at least to the stage reached normally at ten weeks, and in some respects perhaps even beyond it.

These observations will suffice to establish the fact that the skeletal growth during constant body-weight is accompanied by normal developmental changes, as well as changes in chemical composition (percentage of water). In other words, we find not only increase in mass but growth and differentiation apparently normal in character, though somewhat retarded in rate. These skeletal characters therefore tend to correlation with age, although influenced also by the general body-weight.

The remarkable fact that the skeleton continues to grow while the body-weight is held constant was apparently first observed by Waters ('08) who found that calves previously well nourished will continue to increase in height and in width of hip for a considerable time, even when increase of body-weight is prevented by under-feeding. He remarks ('08 b, p. 9):

Apparently the animal organism is capable of drawing upon its reserve for the purposes of sustaining the growth process, for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a certain extent to support growth, the process ceases and there is no further increase in height or in length of bone. From this point on, the animal's chief business seems to be to sustain life. This law applies to animals on a stationary live weight as well as to those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance, and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, get thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.

Aron ('11) experimented with dogs to determine the effect of a restricted amount of food upon young, growing animals. He

found that in spite of constant live weight, the animals continued to increase in length and height for three to five months. Thereupon the emaciated animals became weaker and died unless the amount of food was somewhat increased. From a comparative study of nine bones (the entire skeleton was not measured), Aron concludes that the skeleton during constant body-weight increases in mass and also changes in chemical composition (increase in water-content and protein (?); decrease in fat). The results of this very interesting investigation, while sufficient to establish the continued growth of the skeleton, would be more conclusive if the number of observations were larger, with an adequate number of controls at the beginning and at the end of the experiment. In a recent paper, Aron ('14) records a few observations indicating that malnutrition in children retards growth in length less than body-weight; so that the body may continue to increase in length while the body-weight is at a standstill, or even slightly decreasing. Thus the strong growth tendency of the skeleton during bare maintenance of the body-weight is manifest in the human species, as well as in the calves, dogs and rats.

#### MUSCULATURE

Although the musculature (table 8; fig. 4) in the normal rat at three weeks averages 26.9 per cent of the body, according to Jackson and Lowrey ('12), the controls in the present series gave a somewhat higher amount, the average being 31.2 per cent. As shown in table 8, the musculature in the controls of the present series also averaged slightly higher than the normal according to Jackson and Lowrey at six and ten weeks. In rats held at constant body-weight from the age of three weeks to six, ten and thirteen weeks, the musculature appears relatively very slightly higher than in the controls at three weeks. The apparent increase from three to ten weeks is from 7.40 grams (7.81 grams, less correction corresponding to the smaller body-weight at 10 weeks) to 7.62 grams, or an increase of 3.0 per cent in absolute weight. In the three to sixteen weeks experiment,

TABLE 8

*The musculature; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson and Lowrey).....	13	24.8	6.67	26.9 (20.1-30.2)
Controls at 3 weeks.....	10	25.1	7.81 (6.90-9.82)	31.2 (29.5-35.3)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	8	22.2	7.04 (5.51-8.05)	31.8 (25.1-34.7)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	3	20.2	6.46 (5.68-7.70)	32.1 (31.3-33.2)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	22	23.8	7.62 (4.58-10.87)	32.0 (24.8-36.4)
Body-weight constant 10 weeks (age of 3 to 13 weeks).....	1	25.5	7.90	31.0
Body-weight constant 13 weeks (age of 3 to 16 weeks).....	1	26.0	7.50	29.4
Normal at 6 weeks (Jackson and Lowrey).....	14	64.4	21.10	32.7 (26.1-35.3)
Controls at 6 weeks.....	2	42.4	14.90 (14.85-15.0)	35.3 (35.0-35.6)
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	16.90 (15.30-18.5)	35.7 (34.8-36.6)
Normal at 10 weeks (Jackson and Lowrey).....	10	134.0	55.10	41.1 (37.4-49.1)
Controls at 10 weeks.....	6	135.0	55.80 (44.00-69.2)	41.6 (39.3-44.5)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	31.20 (30.10-33.9)	40.1 (39.7-40.7)
Controls at 32 and 35 weeks.....	6	189.5	81.20 (64.80-119.7)	42.6 (39.0-50.3)

TABLE 9

*Viscera and remainder; average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	RELATIVE WEIGHT OF VISCERA (AND RANGE) PER CENT	RELATIVE WEIGHT OF 'REMAINDER' (AND RANGE) PER CENT
Normal at 3 weeks (Jackson and Lowrey).....	13	24.8	21.3 (20.1-24.8)	12.9 (4.0-19.9)
Controls at 3 weeks.....	10	25.1	20.5 (17.9-24.8)	10.5 (2.6-15.6)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	25.0 (20.7-29.2)	13.6 (7.1-21.6)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	26.8 (25.0-28.5)	2.3 (1.9-2.7)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	22.2 (19.4-26.0)	10.0 (2.5-16.9)
Normal at 6 weeks (Jackson and Lowrey).....	14	64.5	20.4 (18.4-22.9)	12.0 (6.5-17.1)
Controls at 6 weeks.....	1	42.4	19.6	19.1
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	19.5 (19.1-19.9)	16.4 (16.1-16.7)
Normal at 10 weeks (Jackson and Lowrey).....	10	130.5	16.0 (14.9-17.2)	12.5 (1.2-18.4)
Controls at 10 weeks.....	6	135.0	14.3 (13.0-15.7)	13.6 (9.7-16.4)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	16.3 (16.1-16.8)	12.4 (9.7-13.9)
Controls at 32 and 35 weeks.....	5	179.5	12.9 (11.9-13.5)	17.0 (13.7-19.5)

the average is slightly lower. In the six to thirty-two weeks experiment, the musculature appears very slightly higher than in the controls at six weeks, while in the ten to thirty-five weeks experiment, the musculature appears slightly lower than in the controls at ten weeks. In the latter case, however, the controls are too heavy for comparison with those under experiment.

In general, it seems clear from the foregoing that in young rats held at constant body-weight the musculature also remains nearly constant in weight, with perhaps a very slight tendency to increase in the majority of cases. In the course of normal growth during this period, the musculature shows a more rapid growth than any other system, increasing from about 27 per cent of the body at three weeks to 41 per cent at ten weeks of age (Jackson and Lowrey). During inanition in adult rats, the musculature loses approximately in proportion to the entire body, slightly less in acute inanition and slightly more in chronic inanition (Jackson '15 c).

Aron ('11) did not weigh the muscles in his experiments on dogs, but infers (p. 29) that: "Only the flesh, muscles and fat of the body remain as the tissues which must have lost during the course of the experiments." From an analysis of samples taken from the leg muscles, he also concludes that "The muscles contained only one-half of the normal amount of solids," the protein being greatly decreased and the water-content increased. Again, however, his comparison is with controls at the *end* rather than the *beginning* of the experiment, so that no conclusion can be drawn as to the changes taking place during the experiment in the animals held at constant body-weight.

#### VISCERA AND 'REMAINDER'

With the visceral group (table 9; fig. 4) have been included the brain, spinal cord and eyeballs, as well as the thoracic and abdominal viscera. According to Jackson and Lowrey '12, this group decreases from about 21 per cent of the body at three weeks to about 16 per cent at ten weeks of age. This is in fairly close agreement with the controls in the present series, except at

ten weeks. In this case the controls are considerably too heavy for direct comparison with the animals under experiment, which accounts for the discrepancy.

In the animals held at constant body-weight from the age of three weeks to the ages of six, eight and ten weeks, the visceral group shows a distinct increase in weight. This is more marked at six and eight than at ten weeks, which perhaps indicates that the viscera may increase in the earlier part of the experiment, and lose weight later. The experiment from six to thirty-two weeks indicates no essential change in the weight of the viscera. From ten to thirty-five weeks there is a slight gain.

On the whole, it may be concluded that during constant body-weight in young albino rats the visceral group as a whole undergoes but little change in weight, with a slight tendency to increase, especially in the earlier periods. As will be seen later, however, the individual viscera differ greatly in their reactions.

Aron ('11) concludes that in young dogs held at nearly constant body-weight the organs in general do not lose weight. On account of the small number of observations, however, and the lack of adequate controls, it is difficult to draw any satisfactory conclusion from his observations upon the viscera.

The 'remainder' is obtained by deducting from the net body-weight the weight of the integument, skeleton, musculature and viscera. It therefore includes loss by evaporation and escape of fluids, as well as a few small unweighed organs and the masses of dissectable fat. The data in table 9 show a considerable variation, as might be expected. On the whole, however, it appears doubtful whether there is any material change in the weight of the 'remainder' in young rats held at constant body-weight for considerable periods. There is undoubtedly a loss in the fat, but this is probably counterbalanced by an increased water-content of the interstitial connective tissues.

## BRAIN

The brain (table 10) in eleven controls at three weeks of age averaged 1.282 grams, or 5.31 per cent of the (net) body-weight. This corresponds fairly closely with Donaldson's ('08) figure for the normal rat of corresponding weight. In the rats held at constant body-weight from the age of three to six and eight weeks, there appears (table 10) to be a relative increase in the brain, especially at eight weeks, where it forms 6.62 per cent of the body. In reality, however, this relative increase is only apparent, and due to the fact that these animals began the experiment at a lower body-weight, corresponding to which the brain is relatively heavier. The (net) body-weights of the two rats at eight weeks were respectively 18.1 grams and 17.8 grams. According to Jackson ('13, p. 22), the brain normally reaches its maximum relative weight of about 6.7 per cent of the body when the body-weight is about 15 grams. Thus the final brain-weight of the rats held at constant body-weight from the age of three to eight weeks of age is almost exactly that to be expected if the brain-weight has remained constant.

TABLE 10

*The brain; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1).....	52	25.0*	1.285	5.14
Controls at 3 weeks.....	11	24.5	1.282 (1.187-1.364)	5.31 (4.14-6.20)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	7	22.1	1.195 (1.035-1.297)	5.44 (4.50-6.33)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	1.183 (1.180-1.186)	6.62 (6.60-6.63)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	1.267 (1.136-1.379)	5.30 (4.38-6.20)
Normal at 6 weeks (Donaldson '08).....	42	45.0*	1.441	3.20
Control at 6 weeks.....	1	42.4	1.373	3.23
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	1.478 (1.459-1.497)	3.14 (2.96-3.32)
Normal at 10 weeks (Donaldson '08).....	34	75.0*	1.559	2.08
Controls at 10 weeks†.....	6	134.0	1.579 (1.512-1.636)	1.21 (0.96-1.44)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	1.646 (1.603-1.723)	2.12 (2.02-2.18)
Controls at 32 and 35 weeks.....	6	189.5	1.777 (1.657-1.890)	0.97 (0.78-1.24)

\* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

The brain weight has also apparently remained nearly constant in the large group held at constant body-weight from three to ten weeks of age. The average absolute weight is slightly less, but almost in correspondence with the body-weight, so that the average *relative* weight, 5.30 per cent, is nearly identical with that of the controls at three weeks, the beginning of the experiment. In terms of absolute weight, there is a very slight apparent decrease from 1.274 grams (1.282 grams, less correction<sup>3</sup> for difference in body-weight, which averages 24.5 grams in the three weeks controls and 24.0 grams at ten weeks) to 1.267 grams, a decrease of about 0.5 per cent in absolute weight.

In the series held at constant body-weight from the age of six to thirty-two weeks, there is an apparent slight decrease in the brain from about 3.23 per cent to 3.14 per cent of the body, and in the ten to thirty-five weeks series a slight increase (from 2.08 to 2.12 per cent). Considering the small number of observations and the normal variation, however, these apparent differences do not appear to be significant. I would, therefore, conclude from the data above cited that there is probably no appreciable change in the weight of the brain in young albino rats held at constant body-weight for considerable periods.

Hatai ('04) experimented with a series of young rats with initial body-weights corresponding roughly to those of mine at the ages of six to ten weeks. By giving an unfavorable diet (starch and beef-fat) their body-weight was reduced on the average about 30 per cent. The brain in these cases had apparently lost in absolute weight, the average loss being about 5 per cent. These results, however, are of course not directly comparable with those in which the body-weight has remained constant.

In a later experiment, Hatai ('08) by underfeeding with unfavorable diet retarded the growth of a series of five rats, begin-

<sup>3</sup> It should be noted here as in other cases that the correction for organ-weight is not in exact proportion to the difference in body-weight. Allowance must be made for the change in the *relative* weight of the organ corresponding to the change in body-weight.

ning at the age of 30 days, so that at 170 days their average weight was only 91.5 grams, while full-fed controls averaged 146.5 grams. By comparison with 'second controls,' younger rats of body-weight similar to the final weight of the stunted series, he found that in the stunted rats the brain-weight was practically identical with that of normal rats of the same body-weight. In other words, the growth in brain-weight had been retarded in the same proportion as the body-weight. On this principle, if the body-weight were retarded so as to permit no growth at all, that is held at constant weight, we should expect practically no increase in weight of the brain. This is in agreement with my results, as above stated.

More recently Donaldson ('11) has experimented with a larger series (twenty-two litters) of rats held at nearly constant weight (34 grams) from the age of thirty to the age of fifty-one days. In the rats held at constant body-weight, the brain weight averaged 7.7 per cent less than in full-fed controls of the same litters. No direct controls were taken at the *beginning* of the experiment, but from the normal growth formula it is estimated that the initial brain-weight was slightly less than that found in the retarded rats at the end of the experiment. This would indicate an increase of 3.6 per cent in the brain-weight, while the body-weight was held constant. The large number of observations lends weight to this conclusion, although it would be strengthened if direct controls were available at the beginning of the experiment.

It may be noted that if Donaldson's normal (Wistar reference tables) rather than the direct controls be taken as the basis for estimating the initial brain-weight in my three to ten weeks series, the result would indicate a gain similar to that found by Donaldson in his series. On the whole, therefore, we may safely conclude that there is but very slight if any increase in the brain-weight of young albino rats held at constant body-weight for considerable periods of time.

## SPINAL CORD

When the relative weights are compared with the controls at the beginning of the experiment (table 11), or with the theoretical normal according to Donaldson ('08) there appears a very decided increase in the spinal cord at all the age-periods during the experiment. Thus while the body-weight has been held constant from the age of three to that of ten weeks, the spinal cord has apparently increased from an average of 0.179 to 0.243 grams, an increase of about 36 per cent (or slightly more if the initial weight be decreased to correct for the difference of body-weight at three weeks, 24.5 grams, and ten weeks, 24.0 grams). This corresponds to an increase from 0.74 per cent to 1.02 per cent of the net body-weight. The increases at the other age-periods are equally striking.

Donaldson ('11) in the experiments previously mentioned also found an increase in the weight of the spinal cord in rats held at body-weight of about 34 grams from the age of thirty days to that of fifty-one days. He does not estimate this increase exactly but from the normal weight of the cord at the beginning of the experiment (cf. Donaldson '08, table 1) the weight must have increased from about 0.223 to 0.2498 grams, an increase of about 10.7 per cent. While this is not so striking as my results (perhaps in part because my experiments covered a longer period of time) it agrees in indicating during constant body-weight a much stronger growth tendency in the spinal cord than in the brain. This is in agreement with the well-known fact that in general the normal post-natal growth of the spinal cord is relatively much more rapid than that of the brain. This growth of the spinal cord is apparently correlated with the increase in trunk-length (Donaldson).

## EYEBALLS

An increase even more striking than that of the spinal cord is apparent in the eyeballs (table 12). In rats held at constant body-weight from the age of three weeks, the eyeballs increase from a relative weight of about 0.50 per cent of the body-weight to 0.64 per cent at six weeks, 0.82 per cent at eight weeks, and

TABLE 11

*The spinal cord; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Donaldson '08, table 1).....	47	25.0*	0.180	0.72
Controls at 3 weeks.....	11	24.5	0.179 (0.152-0.200)	0.74 (0.56-0.89)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.0	0.207 (0.149-0.235)	0.96 (0.63-1.25)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.192 (0.188-0.195)	1.08 (1.06-1.09)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.243 (0.201-0.314)	1.02 (0.89-1.17)
Normal at 6 weeks (Donaldson '08).....	42	45.0*	0.254	0.57
Controls at 6 weeks.....	1	42.4	0.286	0.67
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.399 (0.378-0.420)	0.85 (0.83-0.86)
Normal at 10 weeks (Donaldson '08).....	32	75.0*	0.333	0.44
Controls at 10 weeks†.....	6	134.0	0.422 (0.341-0.464)	0.32 (0.27-0.39)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.520 (0.500-0.550)	0.67 (0.64-0.69)
Controls at 32 and 35 weeks.....	6	189.5	0.603 (0.491-0.667)	0.33 (0.24-0.44)

\* Gross body-weight.

† Body-weight of controls at 10 weeks too high for comparison.

TABLE 12

*The eyeballs; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	24	21.2	0.105 (0.073-0.125)	0.52 (0.31-0.73)
Controls at 3 weeks.....	10	24.6	0.120 (0.110-0.133)	0.50 (0.34-0.69)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	6	22.1	0.142 (0.138-0.149)	0.64 (0.60-0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	2	18.0	0.146 (0.145-0.146)	0.82 (0.81-0.82)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	19	24.0	0.179 (0.130-0.196)	0.76 (0.57-1.00)
Normal at 6 weeks (Jackson '13).....	42	50.0	0.153 (0.125-0.175)	0.32 (0.18-0.40)
Control at 6 weeks.....	1	42.4	0.152*	0.36
Body-weight constant 26 weeks (age of 6 to 32 weeks)	2	47.1	0.240 (0.230-0.250)	0.51 (0.50-0.52)
Normal at 10 weeks (Jackson '13).....	75.0	0.173	0.23	
Controls at 10 weeks†.....	6	134.0	0.209 (0.195-0.228)	0.16 (0.13-0.21)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	3	77.8	0.275 (0.249-0.323)	0.35 (0.34-0.38)
Controls at 32 and 35 weeks.....	5	184.4	0.260 (0.235-0.296)	0.15 (0.12-0.17)

\* Body-weight of controls at 10 weeks too high for comparison.

0.76 per cent at ten weeks. In terms of absolute weight, the eyeballs have apparently increased from an average of 0.120 grams at three weeks to about 0.179 grams (no correction made for the slight difference in body-weight) at ten weeks, an increase of nearly 50 per cent! In the normal, full-fed rat at ten weeks (average body-weight 112 grams) the eyeballs have reached a weight of only about 0.201 grams (Jackson '13). At this rate, the weight of the eyeballs at a normal body-weight of 75 grams (the body-weight indicated in table 12 as 'normal at 10 weeks' to correspond to the body-weight of the animals held at constant body-weight from the age of ten to thirty-five weeks) would be only about 0.173 grams, or slightly less than that actually reached in the series held at constant body-weight of 24 grams from three weeks to ten weeks of age. The growth of the eyeballs in rats held constant from the age of six to thirty-two weeks, and from ten to thirty-five weeks, is equally striking.

No data upon the growth of the eyeballs under these conditions have been found in the literature. I have shown elsewhere (Jackson '15 a, '15 c), however, that the eyeballs lose but very little if any during inanition in the adult albino rat.

In connection with the astonishing growth capacity of the eyeballs in young animals at constant body-weight, the possibility that the growth of the eyeballs is somewhat independent of that in the body as a whole may be considered, which I have already pointed out (Jackson '13, p. 24). When the large water-content of the eyeballs is considered (85.6 per cent in the rat at twenty days, according to Lowrey '13), it is, after all, not difficult to comprehend the possibility of its continued growth, largely by water-absorption, when growth in the body as a whole is at a standstill.

#### THYROID GLAND

In young albino rats held at constant body-weight from the age of three weeks to six weeks, eight weeks and ten weeks, there is usually a well-marked loss of weight in the thyroid gland (table 13). In the largest group, three to ten weeks, the thyroid has apparently decreased on the average from about 0.033 per cent

TABLE 13

*The thyroid gland; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	26	18.7	0.0060 (0.0034-0.0104)	0.030 (0.018-0.041)
Controls at 3 weeks.....	11	24.6	0.0078 (0.0054-0.0094)	0.033 (0.022-0.042)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	3	23.1	0.0053 (0.0052-0.0056)	0.023 (0.022-0.024)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	0.0058 (0.0047-0.0068)	0.032 (0.026-0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	18	24.1	0.0059 (0.0034-0.0087)	0.025 (0.016-0.036)
Normal at 10 weeks (Jackson '13).....	24	110.0	0.0165 (0.0100-0.030)	0.015 (0.008-0.022)

of the body to 0.025 per cent. Or, in terms of absolute weight, it has decreased from 0.0078 to 0.0059 grams, a decrease of 24 per cent. (A slight correction should be made on account of difference in body-weight.) No observations were made upon the thyroid gland in the experiments beginning at later ages.

During acute inanition in adult rats, the thyroid gland apparently loses little or no weight; while in chronic inanition with an average loss in body-weight of about 36 per cent, the thyroid gland loses only about 22 per cent in weight (Jackson '15). There is some uncertainty as to the exact figures, however, on account of variability and difficulty in dissecting out the thyroid gland in an accurate manner. The same, of course, holds true for the present series.

#### THYMUS

The normal thymus (table 14) at three weeks forms 0.37 per cent of the net body-weight. This decreases, in rats held at constant body-weight, to 0.075 per cent at six weeks of age, to 0.030 (exceptional?) at eight weeks, and to 0.040 per cent at ten weeks. In terms of absolute weight, the thymus has decreased from 0.091 grams at three weeks to 0.017 grams (loss of 81 per cent) at six weeks, and to 0.0094 grams (loss of 90 per cent) at 10 weeks. No correction for the slight difference in body-weight has been made in these estimates.

Normally at ten weeks of age the weight of the thymus should have increased to about 0.24 grams (0.30 grams in the controls).

A maximum absolute weight of about 0.29 grams is reached by the average normal thymus about the age of 85 days (Hatai '14). At one year, it has undergone a complete age-involution, and forms only 0.02 per cent of the body-weight (Jackson '13).

That the weight and structure of the thymus are markedly affected by various adverse conditions has long been known, and the process of involution has recently been thoroughly investigated by Hammar and his pupils. Jonson ('09) experimented with young rabbits subjected to acute and chronic inanition. In the latter case, the diet was restricted so as to maintain the young rabbits at constant body-weight (similar to the present experiment with rats). Under these conditions, Jonson found the weight-curve of the thymus similar to that of the body-fat, although during *acute* inanition the fat decreases somewhat more rapidly. In young rabbits held at constant body-weight the thymus in four weeks is reduced to about one-thirtieth of its initial weight. The cortex suffers the greatest loss, being reduced to one-twelfth of its initial weight within two weeks of chronic inanition at constant body-weight. It would therefore appear that the process of involution is much more rapid and complete in young rabbits than in young rats at the ages included in the present investigation. In both cases, however, the weight of the thymus in hunger involution decreases most rapidly in the earlier weeks of the experiment.

#### HEART

The heart (table 15) in the albino rats held at constant body-weight from the age of three weeks appears to have remained practically constant at about 0.70 per cent of the net body-weight up to the age of ten weeks. In absolute weight, the heart would apparently decrease from 0.167 grams (0.170 grams, less correction for difference in body-weight) to 0.166 grams, a decrease of about 0.6 per cent, which is probably within the limits of experimental error. The very slight decrease at six weeks and increase at eight weeks are also probably not significant. Similarly in the rats held at constant body-weight from six to thirty-

two weeks and from ten to thirty-five weeks the heart has apparently retained almost exactly its initial relative weight. The absolute weight therefore apparently remains unchanged (the differences in the table being due to different initial body-weights).

During inanition in adult rats (Jackson '15 a, '15 c) the heart likewise maintains its relative weight, losing in absolute weight nearly in proportion to the entire body (slightly more in chronic than in acute inanition).

TABLE 14

*The thymus; average absolute weight, average percentage weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	49	18.7	0.071 (0.034-0.135)	0.37 (0.23-0.55)
Controls at 3 weeks.....	11	24.5	0.091 (0.042-0.123)	0.37 (0.22-0.51)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	4	22.7	0.017 (0.011-0.022)	0.075 (0.046-0.101)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	0.0054 (0.0037-0.0071)	0.030 (0.021-0.040)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	0.0094 (0.0054-0.0170)	0.040 (0.019-0.062)
Normal at 6 weeks (Jackson '13).....	42	50.0	0.108 (0.052-0.234)	0.21 (0.14-0.36)
Normal at 10 weeks (Jackson '13).....	42	107.2	0.24 (0.12-0.44)	0.23 (0.13-0.35)
Controls at 10 weeks.....	6	134.0	0.30 (0.25-0.34)	0.23 (0.16-0.29)

TABLE 15

*The heart; average absolute weight; average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	49	18.7	0.135 (0.082-0.250)	0.72 (0.56-0.93)
Controls at 3 weeks.....	11	24.5	0.170 (0.130-0.245)	0.70 (0.55-0.84)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	0.151 (0.123-0.187)	0.68 (0.63-0.85)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	0.129 (0.128-0.130)	0.72 (0.72-0.72)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	0.166 (0.124-0.213)	0.70 (0.55-0.83)
Normal at 6 weeks (Jackson '13).....	42	50.0	0.277 (0.183-0.535)	0.55 (0.44-0.68)
Controls at 6 weeks.....	1	42.4	0.237	0.56
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	0.271 (0.255-0.288)	0.57 (0.57-0.58)
Normal at 10 weeks (Jackson '13).....		75.0	0.375	0.50
Controls at 10 weeks*.....	6	134.0	0.562 (0.489-0.687)	0.44 (0.40-0.48)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	0.389 (0.348-0.450)	0.50 (0.47-0.52)
Controls at 32 and 35 weeks.....	6	189.5	0.873 (0.688-1.29)	0.46 (0.37-0.56)

\* Body-weight of controls at 10 weeks too high for comparison.

## LUNGS

The lungs (table 16) in rats held at constant body-weight from the age of three weeks to the ages of six weeks and ten weeks appear to lose in weight. (The data at eight weeks are abnormally high, as noted in the table). Thus between the ages of three weeks and ten weeks, the lungs apparently decrease from 1.04 per cent to 0.91 per cent of the body-weight: or in absolute weight from 0.250 to 0.218 grams, a loss of about 15 per cent. (A slight correction has been made on account of the difference in body-weight). In the few observations upon rats for longer periods beginning at the later ages of six and ten weeks, there appears to be no material change in the weights of the lungs during the experimental periods.

The lung infections frequently found in older and adult rats, rarely occur before the age of ten weeks, and thus do not affect the present series. As Hatai has already noted, rats during chronic inanition appear to be unusually free from lung infection.

During inanition in adult rats, the lungs lose weight in about the same proportion as the whole body, thus nearly maintaining their relative (percentage) weight. The loss is slightly greater, however, during chronic inanition (Jackson '15 c).

TABLE 16

*The lungs; average absolute weight; average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT, NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	49	18.7	0.216 (0.151-0.354)	1.17 (0.86-1.46)
Controls at 3 weeks.....	11	24.5	0.253 (0.201-0.290)	1.04 (0.89-1.12)
Body-weight constant 3 weeks (age of 3 to 6 weeks) .....	3	20.9	0.192 (0.190-0.197)	0.92 (0.88-0.93)
Body-weight constant 5 weeks (age of 3 to 8 weeks) .....	2	18.0	0.250* (0.241-0.319)	1.56* (1.36-1.77)
Body-weight constant 7 weeks (age of 3 to 10 weeks) .....	19	24.0	0.218 (0.170-0.282)	0.91 (0.78-1.32)
Normal at 6 weeks (Jackson '13).....	39	50.0	0.333 (0.244-0.547)	0.68 (0.58-0.94)
Control at 6 weeks.....	1	42.4	0.264	0.62
Body-weight constant 26 weeks (age of 6 to 32 weeks) .....	2	47.1	0.319 (0.309-0.329)	0.68 (0.65-0.70)
Normal at 10 weeks (Jackson '13).....		75.0	0.49	0.65
Controls at 10 weeks.....	6	134.0	0.74 (0.610-0.94)	0.56 (0.45-0.74)
Body-weight constant 25 weeks (age of 10 to 35 weeks) .....	3	77.8	0.49 (0.390-0.60)	0.63 (0.52-0.81)

\* Lungs in one case abnormally heavy on account of post mortem congestion.

## LIVER

The liver (table 17) in the albino rats held at constant body-weight from the age of three weeks to six, eight and ten weeks shows an apparent increase. This increase appears greatest in the shortest series, three to six weeks, increasing from 4.95 per cent of the body-weight (in controls) to 5.89 per cent. Between three and ten weeks the corresponding increase is from an absolute weight of 1.16 grams (1.20 grams, less correction for difference in body-weight; or 4.95 per cent of the body-weight) to 1.28 grams (5.25 per cent of the body-weight), or an increase of about 10.3 per cent in absolute weight.

In the later and longer periods, however, there appears to be a decided decrease in the weight of the liver (six to thirty-two weeks and ten to thirty-five weeks series). This may be due to the fact that the latter experiments extended over a longer period of time, or it may be because they were begun at a later age. We may therefore conclude that in young albino rats held at constant body-weight there is, beginning at three weeks, an increase in the weight of the liver (apparently greater at six than at ten weeks of age); while in rats beginning the experiment later, at six and ten weeks of age (and extending over a longer period)

TABLE 17

*The liver; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13) .....	49	18.7	0.87 (0.42-2.08)	4.50 (3.21-5.82)
Controls at 3 weeks .....	11	24.5	1.20 (1.06-1.38)	4.95 (4.03-6.00)
Body-weight constant 3 weeks (age of 3 to 6 weeks) .....	7	22.1	1.30 (0.89-1.63)	5.89 (4.32-7.39)
Body-weight constant 5 weeks (age of 3 to 8 weeks) .....	2	18.0	0.92 (0.78-1.06)	5.12 (4.36-5.88)
Body-weight constant 7 weeks (age of 3 to 10 weeks) .....	19	24.0	1.28 (0.80-1.82)	5.25 (3.63-7.00)
Normal at 6 weeks (Jackson '13) .....	42	50.0	3.19 (2.18-4.76)	6.48 (5.39-8.46)
Control at 6 weeks .....	1	42.4	2.18	5.15
Body-weight constant 26 weeks (age of 6 to 32 weeks) .....	2	47.1	2.33 (2.21-2.45)	4.44
Normal at 10 weeks (Jackson '13) .....		75.0	4.50	6.00
Controls at 10 weeks .....	6	134.0	6.72 (4.91-8.66)	4.99 (4.28-5.72)
Body-weight constant 25 weeks (age of 10 to 35 weeks) .....	3	77.8	3.11 (2.30-3.51)	4.25 (3.91-4.74)
Controls at 32 and 35 weeks .....	6	189.5	7.27 (5.84-9.86)	3.84 (3.47-4.14)

there is a decided decrease in the weight of the liver. Thus the liver would appear under these conditions to have a tendency to follow the normal growth-impulse, which increases to a maximum at about the age of six weeks, and decreases thereafter (Jackson '13, p. 31).

During inanition in adult rats, the liver loses in weight relatively more than the whole body, and to a greater extent in acute than in chronic inanition (Jackson '15 c). As the liver is normally subject to great variation in weight, however, (Jackson '13) great caution must be observed in drawing final conclusions. Hatai ('13) found the weight of the normal liver distinctly higher than that in my series, and if his data instead of my controls were taken as a basis for the initial weights, the estimated losses in my experiments would be considerably greater, even the younger rats showing a loss instead of a gain in liver-weight.

#### SPLEEN

Taking the controls at three weeks as a basis for comparison, it appears that in rats held at constant body-weight from the age of three weeks to six weeks the weight of the spleen (table 18) remains practically unchanged (the average at six

TABLE 18

*The spleen; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	49	18.7	0.055 (0.019-0.145)	0.28 (0.15-0.42)
Controls at 3 weeks.....	11	24.5	0.091 (0.051-0.130)	0.37 (0.27-0.48)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	0.087 (0.040-0.172)	0.38 (0.18-0.72)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	0.043 (0.036-0.049)	0.24 (0.20-0.27)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	0.053 (0.033-0.076)	0.22 (0.16-0.33)
Normal at 6 weeks (Jackson '13) .....	42	50.0	0.135 (0.085-0.204)	0.28 (0.19-0.47)
Control at 6 weeks.....	1	42.4	0.107	0.25
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	0.139	0.29 (0.28-0.30)
Normal at 10 weeks (Jackson '13) .....		75.0	0.225	0.30
Controls at 10 weeks.....	6	134.0	0.350 (0.300-0.420)	0.27 (0.21-0.36)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	0.230 (0.230-0.240)	0.30 (0.27-0.33)
Controls at 32 and 35 weeks.....	6	180.5	0.620 (0.480-0.750)	0.33 (0.29-0.39)

weeks being probably too high, on account of the inclusion of a litter with abnormally large spleens); while in the rats at eight and ten weeks there is a considerable decrease. In the case of the three to ten weeks series, the decrease would be from 0.091 gram (0.37 per cent of the net body-weight) to 0.053 gram (0.22 per cent of the body-weight), or a decrease of nearly 42 per cent in absolute weight. (No correction has been made for the difference in body-weight, which is 0.5 gram lower at ten weeks). If the normal relative weight of the spleen at three weeks (0.28 per cent, Jackson '13) be taken as a basis of estimate, however, the loss would appear considerably less.

In the rats held at constant body-weight at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there appears to be no appreciable change in the average weight of the spleen. It may be concluded therefore that in young rats held at constant body-weight beginning at the age of three weeks there is a marked tendency to a reduction in the weight of the spleen; while at later (and longer) periods the spleen appears to undergo no material change in weight. It must be remembered, however, that the spleen is normally one of the most variable organs in the body (Jackson '13), and final conclusions should be correspondingly guarded.

In adult albino rats during chronic inanition the average loss in weight is nearly proportional to that of the entire body, while in acute inanition the loss appears very much greater (Jackson '15 a, '15 c).

#### STOMACH AND INTESTINES

The stomach and intestines, including mesentery and pancreas, are considered both with contents (table 19 a) and empty (table 19 b). Considering first the empty canal, it appears in rats held at constant body-weight from the age of three weeks to increase from about 4.8 per cent of the body-weight to 8.0 per cent at six and eight weeks, decreasing to 6.0 per cent at ten weeks. In absolute weight the increase would be from about 1.13 grams (1.20 grams less correction on account of difference in body-weight) at three weeks to 1.45 grams at ten weeks, an

increase of about 28 per cent. Between six and thirty-two weeks there is apparently but little change; while from ten to thirty-five weeks there appears to be a decrease in the weight of the alimentary canal. The number of observations, however, is too small for final conclusion.

The most remarkable increase occurs between three and six weeks of age, from an absolute weight of 1.20 to 1.78 grams, an apparent increase of about 48 per cent in absolute weight! (The increase would be even greater if allowance were made for difference in body-weight). Thus, as in the case of the liver and

TABLE 19

*The stomach and intestines; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
<i>a. Including contents</i>				
Normal at 3 weeks (Jackson '13).....	49	18.7	1.78 (0.74-3.08)	9.3 (5.5-15.5)
Controls at 3 weeks.....	11	24.5	2.51 (1.96-3.23)	10.4 (7.8-15.4)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	3.10 (1.67-5.07)	13.9 (7.4-21.3)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	3.64 (2.58-4.71)	20.2 (4.5-26.0)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	3.29 (2.26-6.83)	13.5 (10.3-20.2)
Normal at 6 weeks (Jackson '13) .....	42	50.0	8.05 (4.48-13.40)	15.9 (10.6-23.4)
Controls at 6 weeks.....	1	42.4	5.13	12.1
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	5.98 (4.71-7.24)	12.5 (10.8-14.2)
Normal at 10 weeks (Jackson '13) .....		75.0	9.00	12.0
Controls at 10 weeks*.....	6	134.0	11.32 (8.82-14.49)	8.3 (7.3-11.2)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	7.48 (5.90-8.81)	9.6 (8.1-10.4)
Controls at 32 and 35 weeks.....	6	189.5	11.60 (7.51-14.04)	7.4 (5.4-12.2)
<i>b. Empty</i>				
Normal at 3 weeks (Jackson '13).....	16	20.0	0.90 (0.37-1.61)	4.5 (2.9-6.1)
Controls at 3 weeks.....	10	25.1	1.20 (0.74-1.69)	4.8 (3.1-6.7)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	1.78 (1.41-2.51)	8.0 (6.3-11.0)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	1.44 (1.38-1.51)	8.0 (7.8-8.3)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	1.45 (0.96-2.29)	6.0 (4.5-8.6)
Normal at 6 weeks (Jackson '13).....		50.0	4.00	8.0
Controls at 6 weeks.....	1	42.4	3.00	7.1
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	3.22 (2.85-3.59)	6.8 (6.5-7.0)
Normal at 10 weeks (Jackson '13).....		75.0	5.30	7.0
Controls at 10 weeks*.....	5	127.9	5.26	4.3 (3.9-4.9)
Body-weight constant 25 weeks (age of 10 to 25 weeks).....	3	77.8	3.97 (3.48-4.35)	5.4 (4.7-5.9)
Controls at 32 and 35 weeks.....	6	189.5	9.16 (7.57-10.64)	4.9 (4.1-5.3)

\* Body-weight of controls at 10 weeks too great for comparison with those held constant 10-35 weeks.

following the normal growth tendency, there appears to be in these retarded rats an early increase in the weight of the alimentary canal, reaching a maximum at about the age of six weeks, after which there is a decline in weight. It may be that the early increase in these organs is diminished later on account of the exhaustion of the stored food-supplies available elsewhere in the body at the beginning of the experiment. Individual organs and tissues differ greatly from each other in their relative susceptibility to attack and absorption at different times during the course of inanition.

The behavior of the stomach and intestines weighed with contents (table 19 a) is very similar to that of the empty canal. Contrary to what might be expected, during constant body-weight, with a restricted food-supply (water *ad libitum*), the canal does not decrease in contents. On the contrary there is an *increase* in contents (watery or mucous in character) which may even exceed in relative weight the normal contents in full-fed animals. The maximum occurred in animals held at constant body-weight from the age of three to eight weeks, when the canal with contents formed 20.2 per cent of the body-weight! In experiments beginning at later ages and extending over longer periods (six to thirty-two weeks and ten to thirty-five weeks), the canal with contents appears to remain more nearly uniform in weight, with some tendency to decrease.

During both acute and chronic inanition in adult albino rats, there is a very marked decrease in the weight of the stomach and intestines, both with and without contents (Jackson '15 a, '15 c).

#### SUPRARENAL GLANDS

The suprarenal glands (table 20) from the age of about six weeks must be considered separately in the sexes, on account of a distinct sexual difference in their weight, as discovered independently by Hatai ('13) and myself (Jackson '13). In the earlier ages, however, there is no apparent sexual difference, hence the sexes are combined.

TABLE 20

*The suprarenal glands; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks* (Jackson '13).....	49	18.7	0.0072 (0.0040-0.0140)	0.0400 (0.023-0.074)
Controls at 3 weeks*.....	11	24.5	0.0088 (0.0060-0.0108)	0.0370 (0.025-0.055)
Body-weight constant 3 weeks* (age of 3 to 6 weeks).....	7	22.1	0.0089 (0.0070-0.0110)	0.0400 (0.034-0.050)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	1m 1f	18.1 17.8	0.0109 0.0088	0.0610 0.0490
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	7m 12f	25.2 23.3	0.0101 (0.0072-0.0126) 0.0117 (0.0090-0.0147)	0.0420 (0.033-0.052) 0.0510 (0.040-0.061)
Normal at 6 weeks* (Jackson '13).....	42	50.0	0.0128 (0.0070-0.0190)	0.0260 (0.015-0.039)
Control at 6 weeks.....	1f	42.4	0.0090	0.0210
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	1m 1f	43.7 50.5	0.0105 0.0090	0.0240 0.0180
Normal at 10 weeks (Jackson '13).....	20m 23f	117.0 99.0	0.0220 (0.0150-0.0336) 0.0253 (0.0180-0.0329)	0.0185 (0.011-0.025) 0.0257 (0.017-0.032)
Controls at 10 weeks.....	3m 3f	154.7 114.0	0.0295 (0.0270-0.0326) 0.0318 (0.0290-0.0346)	0.0190 (0.017-0.023) 0.0280 (0.027-0.031)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	2m 1f	79.6 74.2	0.0149 (0.0147-0.0150) 0.0145	0.0190 (0.017-0.020) 0.020
Controls at 32 and 35 weeks.....	3m 3f	218.7 160.3	0.0270 (0.0180-0.0310) 0.0320 (0.0280-0.0360)	0.0100 (0.009-0.013) 0.0200 (0.017-0.024)

\* No sexual difference apparent.

TABLE 21

*The kidneys; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	49	18.7	0.271 (0.169-0.531)	1.44 (1.19-1.87)
Controls at 3 weeks.....	11	24.5	0.393 (0.314-0.487)	1.62 (1.33-2.16)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	7	22.1	0.396 (0.328-0.487)	1.80 (1.46-2.05)
Body-weight constant 5 weeks (age of 3 to 8 weeks).....	2	18.0	0.339 (0.331-0.347)	1.89 (1.86-1.92)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	19	24.0	0.404 (0.325-0.515)	1.69 (1.44-1.97)
Normal at 6 weeks (Jackson '13).....	42	50.0	0.616 (0.500-0.943)	1.25 (1.00-1.55)
Controls at 6 weeks.....	1	42.4	0.370	1.35
Body-weight constant 26 weeks (age of 6 to 32 weeks).....	2	47.1	0.613 (0.581-0.645)	1.30 (1.28-1.33)
Normal at 10 weeks (Jackson '13).....		75.0	0.750	1.00
Controls at 10 weeks*.....	6	134.0	1.200 (0.953-1.481)	0.91 (0.81-0.99)
Body-weight constant 25 weeks (age of 10 to 35 weeks).....	3	77.8	0.753 (0.728-0.784)	0.97 (0.92-1.02)
Controls at 32 and 35 weeks.....	6	189.5	1.539 (1.275-1.909)	0.81 (0.71-0.86)

\* Controls too heavy for comparison.

In the rats held at constant body-weight from the age of three weeks the suprarenal glands appear to increase slightly in weight at six and eight weeks; and especially at ten weeks, where the characteristic sexual difference has distinctly appeared. In absolute weight, the suprarenal glands have increased from 0.0090 gram (0.0088 gram, plus correction for difference in body-weight; or 0.037 per cent of the body-weight) at three weeks to 0.0101 gram (0.042 per cent of the body-weight) in the male at ten weeks, an increase of about 12 per cent in absolute weight. In the female, the corresponding increase is from 0.0084 gram (0.0088 gram, less correction for difference in body-weight) (or 0.037 per cent of the body-weight) to 0.0117 gram (0.051 per cent of the body-weight), an increase of 39 per cent in absolute weight. Normally the suprarenals during this period are decreasing in relative (percentage) weight. In the experiments at later and longer periods (six to thirty-two weeks and ten to thirty-five weeks), the changes are apparently not great, but a larger number of observations is necessary before definite conclusions can be reached.

In adult rats during inanition there is little or no loss in the absolute weight of the suprarenal glands, which therefore increase markedly in relative (percentage) weight (Jackson '15 a, '15 c).

#### KIDNEYS

The kidneys (table 21) in rats held at constant body-weight from the age of three weeks show a tendency to increase which is more marked at six and eight than at ten weeks. Between three and ten weeks of age the increase is from an average of 0.388 gram (0.393 gram, less correction on account of difference in body-weight; or 1.62 per cent of the body-weight) to 0.404 gram (1.69 per cent of the body-weight), an increase of only about 4.1 per cent in absolute weight. At later and longer periods (six to thirty-two weeks and ten to thirty-five weeks) there is apparently but little change in the weight of the kidneys. The slight differences shown in the table are probably not significant. On the whole, it appears that in young rats held at

constant body-weight there is a slight tendency to increase in the weight of the kidneys, in the earlier weeks, but little or no apparent difference later.

In adult rats during acute and chronic inanition, the kidneys lose in weight relatively slightly less than the body as a whole, thereby gaining slightly in relative (percentage) weight (Jackson '15 c).

#### TESTES AND EPIDIDYMI

The weights of testes and epididymi unfortunately were not separated in some cases, which (together with their variability and the small number of observations) makes conclusions somewhat difficult. For the testis (table 22 a), the clearest case is in rats held constant from three to ten weeks of age, in which

TABLE 22

*The testes and epididymi; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
<i>a. Testes</i>				
Normal at 3 weeks (Hatai '13).....		20.0*	0.090	0.45
Normal at 3 weeks (incl. epididymi) (Jackson '13)	24	21.2	0.134† (0.076-0.224)	0.63* (0.53-0.78)
Controls at 3 weeks.....	6	25.0	0.144 (0.114-0.200)	0.57 (0.49-0.62)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	2	22.1	0.133† (0.129-0.137)	0.66* (0.65-0.67)
Body-weight constant 5 weeks (age of 3 to 8 weeks)	1	18.1	0.176†	0.98*
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.193 (0.106-0.331)	0.74 (0.49-1.05)
Normal at 6 weeks (Hatai '13).....		50.0*	0.402	0.80
Normal at 6 weeks (Jackson '13).....	20	49.0	0.592† (0.365-0.958)	1.19* (0.88-1.72)
Body-weight constant 26 weeks (age of 6 to 32 weeks)	1	43.7	0.370†	0.84*
Normal at 10 weeks (Hatai '13).....		70.0*	0.774	1.11
Normal at 10 weeks (Jackson '13).....	20	117.0	1.747† (0.678-2.62)	1.51* (0.63-2.41)
Controls at 10 weeks.....	3	154.7	1.850 (1.608-2.10)	1.21* (1.06-1.49)
Body-weight constant 25 weeks (age of 10 to 35 weeks)	2	79.6	1.294† (1.401-1.188)	1.62* (1.61-1.64)
Controls at 32 and 35 weeks.....	3	218.7	1.844† (1.766-1.932)	0.84* (0.74-0.90)
<i>b. Epididymy</i>				
Normal at 3 weeks (Jackson '13)..... (estimated)		20.0	0.200 (?)	0.10 (?)
Controls at 3 weeks.....	6	25.0	0.0209 (0.0092-0.0246)	0.084 (0.039-0.123)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	1	23.6	0.0144	0.061
Body-weight constant 7 weeks (age of 3 to 10 weeks)	7	25.2	0.0209 (0.0084-0.0360)	0.080 (0.040-0.115)
Controls at 10 weeks.....	3	154.7	0.4700 (0.4480-0.4920)	0.310 (0.290-0.330)

\* Gross body-weight.

† Including epididymy.

there is an increase from 0.144 gram (0.57 per cent of the body-weight) to 0.193 gram (0.74 per cent of the body), an apparent increase of 34 per cent in the absolute weight. (The average net body-weight was about the same in both cases, 25.0 grams in the controls, and 25.2 grams at ten weeks).

For the epididymi (table 22 b), the conclusions are even more uncertain; but there appears to be a slight loss in the relative weight of the epididymi in rats held at constant body-weight from the age of three to six and ten weeks.

For testis and epididymis combined (table 22 a) there appears to be an increase in rats held at constant body-weight, excepting the period from six to thirty-two weeks.

While no final conclusions can be drawn, the evidence indicates that in young rats held at constant body-weight there is an increase in the weight of the testis, but not in the epididymis.

During inanition in adult rats, the testes and epididymi apparently lose weight in about the same proportion as the entire body (Jackson '15 c).

#### OVARIES

In rats held at constant body-weight from three weeks to ten weeks of age, there would appear to be a decrease in the weight of the ovaries (table 23) from 0.0066 gram (0.0068 gram, less correction for difference in body-weight; or 0.027 per cent of the

TABLE 23

*The ovaries; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSERVATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE): PER CENT
Normal at 3 weeks (Jackson '13).....	24	16.2	0.0036 (0.0015-0.0067)	0.0220 (0.011-0.039)
Controls at 3 weeks.....	5	24.5	0.0068 (0.0029-0.0104)	0.0270 (0.012-0.034)
Body-weight constant 3 weeks (age of 3 to 6 weeks).....	3	22.4	0.0067 (0.0048-0.0084)	0.0300 (0.020-0.038)
Body-weight constant 7 weeks (age of 3 to 10 weeks).....	12	23.3	0.0048 (0.0030-0.0064)	0.0210 (0.012-0.028)
Normal at 6 weeks (Jackson '13).....	20	54.9	0.0106 (0.0050-0.028)	0.0216 (0.012-0.035)
Normal at 10 weeks (Jackson '13).....	23	98.8	0.0350 (0.0100-0.070)	0.0340 (0.013-0.055)
Controls at 10 weeks.....	3	114.0	0.0237 (0.0297-0.038)	0.0290 (0.027-0.033)

body-weight) to 0.0048 gram (0.021 per cent of the body-weight). This would indicate a decrease of about 27 per cent in absolute weight. The ovary, however, is an organ which (like the thyroid gland) is quite variable and somewhat difficult to dissect out with accuracy, so a much larger number of observations would be required for a final conclusion.

#### HYPOPHYSIS

In the hypophysis (table 24) there is normally a sexual difference in weight, observable in rats above 50 grams in body-weight (Hatai '13). As in the case of the suprarenal glands, the hypophysis normally becomes relatively heavier in the female.

In the rats held at constant body-weight from the age of three weeks to six weeks, there is what appears to be a sexual difference, the one male with a hypophysis of 0.0016 gram (0.0068 per cent of the body-weight), while the two female hypophyses each weigh 0.0018 gram (0.0079 per cent of the body-weight). These are probably mere accidental variations, however, as in the three to ten weeks series the difference in the relative weight is insignificant (0.0084 per cent of the body-weight in the males, and 0.0086 per cent in the females). In any event, however,

TABLE 24

*The hypophysis; average absolute weight, average percentage of net body-weight and range indicated*

DESCRIPTION OF RATS	NO. OF OBSER- VATIONS	BODY-WEIGHT: NET GRAMS	ABSOLUTE WEIGHT (AND RANGE): GRAMS	RELATIVE WEIGHT (AND RANGE) PER CENT
Normal at 3 weeks* (Hatai '13).....		25.0†	0.00175	0.0070
Controls at 3 weeks*.....	9	25.5	0.00178 (0.0012-0.0022)	0.0070 (0.0052-0.0084)
Body-weight constant 3 weeks (age of 3 to 6 weeks)	{ 1m. 2f.	23.6 22.9	0.0016 0.0018	0.0068 0.0079 (0.0076-0.0082)
Body-weight constant 7 weeks (age of 3 to 10 weeks)	{ 7m. 11f.	25.2 23.6	0.0021 (0.0016-0.0024) 0.0020 (0.0010-0.0033)	0.0084 (0.0057-0.0110) 0.0086 (0.0043-0.0135)
Normal at 10 weeks (Hatai '13).....	{ m. f.	120.0 130.0	0.0054 0.0084	0.0042 0.0065
Controls at 10 weeks.....	{ 3m. 3f.	154.7 114.0	0.0062 (0.0059-0.0067) 0.0054 (0.0046-0.0067)	0.0040 (0.0038-0.0042) 0.0048 (0.0040-0.0062)

\* No sexual difference apparent.

† Gross body-weight.

there appears to be a distinct tendency to an increase in the weight of the hypophysis in rats held at constant weight from three to ten weeks of age. In absolute weight, this corresponds in the male to an increase from 0.00178 gram to 0.0021 gram, an increase of about 18 per cent. In the female, the corresponding increase is from 0.00168 gram (0.00178 gram, less correction for difference in body-weight) to 0.0020 gram, an increase of 19 per cent.

As already shown, the suprarenal glands differ from the hypophysis in that they undergo a marked sexual differentiation in weight during their growth while the body-weight is held constant. The impulse to sexual differentiation in these glands therefore appears stronger in the suprarenals than in the hypophysis. Final conclusions should be guarded, however, until a larger number of observations is available.

In the adult rat during inanition the weight of the hypophysis decreases in nearly the same proportion as the body-weight (Jackson '15 c).

#### DISCUSSION

With reference to their growth tendency in young rats held at constant body-weight, the organs may be divided into three classes: (1) those in which the growth tendency is so strong that they continue to increase, even when the body-weight is held constant; (2) those which approximately hold their weight constant under these conditions; and (3) those which are unable to maintain themselves and lose in weight.

According to this scheme, the organs in rats held at constant body-weight from the age of three to that of ten weeks are grouped in table 25. No grouping of this sort can be entirely satisfactory, because in some cases organs are intermediate in position, and especially because (as has been shown) in many cases the weight of an organ will vary according to the age at which the experiment was begun and the length of the period. For example, the liver, which in the three-to-ten weeks experiment shows but a slight gain, shows a larger gain at earlier times, and a loss at later and longer periods.

On the whole, however, the grouping suffices to give a good view of the results. Group I shows the organs with marked growth tendency to be the skeleton, eyeballs, spinal cord, alimentary canal, testes, hypophysis and suprarenal glands. Group II, which approximately maintains constant weight, includes the musculature, brain, heart, kidneys and liver. Group III, those which fail to maintain their weight when the body-weight is held constant, includes the integument, lungs, thyroid gland, ovaries, spleen and thymus.

In the same table 25 for convenience of comparison are also grouped the various organs of the adult rat according to their relative loss in weight during chronic inanition (Jackson '15 c).

TABLE 25

*Comparison of growth tendency in young rats held at constant body-weight from age of three to ten weeks with tendency to maintenance in adult rats during chronic inanition. The figures indicate the apparent average percentage gain or loss in absolute weight during the period of experiment*

	GROUP I		GROUP II		GROUP III	
	Marked tendency to growth in young held at constant body-weight. Strong tendency to maintenance in adult chronic inanition		Weight nearly constant in young held at constant body-weight. Relative loss similar to that of entire body during adult chronic inanition		Marked loss in weight in young held at constant body-weight. Relative loss greater than that of entire body during adult chronic inanition	
Young at constant body weight....	*	per cent of change		per cent of change		per cent of change
	*eyeballs	+50.0	liver	+10.3	lungs	-15.0
	*spinal cord	+36.0	*kidneys	+4.1	thyroid gland	-24.0
	testes	+34.0	*musculature	+3.0	ovaries	-27.0
	*skeleton	+28.0	brain	-0.5	integument	-36.0
	alimentary canal	+28.0	*heart	-0.6	spleen	-42.0
	*suprarenal glands	{ M. +12.0 F. +39.0			thymus	-90.0
	hypophysis	{ M. +18.0 F. +19.0				
Adults during chronic inanition (loss of body-weight 36 per cent)	*skeleton	+1.8	hypophysis	-25.3	liver	-43.0
	*spinal cord	-4.0	*kidneys	-26.8	alimentary canal	-57.0
	*eyeballs	-5.8	spleen	-29.0		
	brain	-6.6	*heart	-32.8		
	*suprarenal glands	-8.9	integument	-38.5		
	thyroid gland	-21.8	lungs	-40.0		
	thymus	(?)	testes	-40.3		
			*musculature	-40.8		

\* Indicates correspondence between the groupings in young and adult series.

This enables us to answer the question as to whether the organs in young animals held at constant body-weight (and thus subjected to a chronic inanition) behave in a manner similar to adult rats during chronic inanition. In many cases, the grouping shows an agreement. The skeleton, eyeballs, spinal cord and suprarenal glands have a strong growth tendency in young held at constant body-weight, and also a strong tendency to maintain their original weight in adults subjected to chronic inanition. The musculature, heart and kidneys approximately maintain their weight in young held constant, and maintain their *relative* weight in adults during chronic inanition. In the majority of cases, however, the behavior of organs in the young differs materially from that in the adult. Thus the alimentary canal has a marked growth in the young at constant weight, yet it loses heavily during adult inanition. The converse is apparently true of the thyroid gland. To a greater or less degree, this inconsistency is seen in the case of most of the organs, as is evident from table 25.

This inconsistency is perhaps to be explained in the following manner. In the adult during inanition the various organs lose weight relatively in inverse ratio to the ability of their cells to extract nutrition from the diminishing quantity available in the surrounding medium and to maintain equilibrium under these adverse conditions. In the young animal held at constant body-weight, the conditions differ in that the corresponding cells have the capacity not only to *Maintain* themselves, but to *grow*. The growth capacity, as is well known, is different from and to some extent independent of the maintenance capacity. The adult has lost the capacity to grow, whereas the young animal has both the powers of growth and maintenance. Hence their organs behave differently during inanition. The alimentary canal, for example, apparently has a strong growth tendency, but a weak power of maintenance.

It is further evident, however, that even the growth capacities of the various tissues and organs differ relatively from each other under different planes of nutrition. Thus under normal conditions of growth in the rat between three and ten weeks of age

the *muscular* system shows the strongest growth capacity, and increases with greater relative rapidity than any other system (Jackson and Lowrey). The skeleton is of course growing steadily, but at a much slower rate, so that it is decreasing in relative (percentage) weight. Under the adverse nutritive conditions in young animals when the body-weight is held constant, however, the musculature is barely able to maintain its weight, while the skeleton is able to absorb more than its share of the available nutrition, and to grow steadily (though at a retarded rate). There are similar differences among many of the individual organs, though in some cases (e.g., liver, alimentary canal) there is a certain degree of parallelism between the normal growth tendency and the behavior when the body-weight is held constant at corresponding periods.

That also the power of maintenance may vary in organs according to the nutritional conditions is shown by the characteristic differences in the losses of organ-weight in chronic inanition as compared with acute inanition in adults (Jackson '15 a, '15 c).

Finally, it should be remembered that the results of the present paper, as well as those concerning adult chronic inanition in a previous paper (Jackson '15 c), are based upon the use of a diet wholesome and balanced, but insufficient in quantity. It is probable that more or less different results would follow from other forms of inanition, such as 'partial inanition' from a chemically defective or highly unbalanced diet. For example, Hatai ('15) finds a pronounced atrophy of the testis and other characteristic changes in albino rats whose growth had been retarded by a 'lipoid-free' ration. Bowin ('80), however, in dogs and rabbits fed dry food only (no water) found, with a loss of about 50 per cent in body-weight, the losses in organ-weight similar to those following total hunger.

## SUMMARY

The principal results of the present paper may be summarized briefly as follows:

Young albino rats may be held at constant body-weight for considerable periods by underfeeding. The amount of food required for this purpose decreases as the experiment proceeds.

As to the body-proportions, the relative weights of the head, trunk and extremities remain practically unchanged during the experiment. There is apparently a slight increase in the head, counterbalanced by a corresponding decrease in the trunk and extremities, but the change is so slight as to seem of doubtful significance.

Of the systems—integument, skeleton, musculature, viscera and 'remainder'—there is but little change in the weights of the musculature, visceral group (as a whole) and 'remainder.' There is, however, a marked decrease in the weight of the integument, counterbalanced by a marked increase in the skeleton. Thus on the low plane of nutrition in the young body maintained at constant weight, the growth capacity appears weakest in the skin and strongest in the skeletal system. This is in striking contrast with the normal growth process of corresponding ages, during which the musculature increases with relatively great rapidity and the skeleton lags behind relatively.

The increase in the skeleton during constant body-weight appears to involve the ligaments as well as the cartilages and bones. The skeletal growth tends to proceed along the lines of normal development, as indicated by decrease in the water-content, and by formation and union of various epiphyses. Another evidence of the tendency to normal development of the skeleton is seen in the increased relative length of the tail as compared with the body-length. The teeth also continue to develop normally (formation and eruption of the third molars).

The individual viscera may be classified in three groups:

(1) There is during the maintenance of constant body-weight in young rats a well-marked increase in the weights of the eye-

balls, spinal cord, alimentary canal (both empty and including contents), testes, hypophysis and suprarenal glands. The suprarenals undergo sexual differentiation in weight (as occurs normally), but the hypophysis apparently does not.

(2) There is no marked change in the weights of the brain, heart, kidneys and epididyma. The liver is variable, showing a definite increase in the earlier periods, but a decrease later. The lungs show a slight decrease in the early periods, but not in later.

(3) There is a well-marked decrease in the weights of the thymus ('hunger involution'), spleen, thyroid gland and ovaries.

When the organs are similarly grouped according to degree of loss during chronic inanition in the adult (slight loss, loss proportional to body, and loss greater than body), many differences are found on comparison with the corresponding groups in the young during constant body-weight. This is explained as due to the presence of both the growth tendency and the (more or less different) maintenance tendency in the young animals, whereas in the adult there is only the tendency to maintenance. Both the growth tendency and the maintenance tendency, however, show characteristic differences in the various organs according to nutritional conditions (normal nutrition, acute or chronic inanition).

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# INHERITANCE IN THE ASEXUAL REPRODUCTION OF HYDRA

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TEN FIGURES

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## I. INTRODUCTION

The theoretical bearing of the work of Johannsen upon pure lines was not widely appreciated by students of heredity for the first few years after its publication; the general applicability of Mendel's law had not been shown and, hence, the genotype conception was without the support since gained from the evi-

dence bearing upon the stability of the unit character. The first attempt to test the general application of Johannsen's conclusions was made by Elise Hanel. In 1908 she published the results of two years of experimental study of inheritance in the asexual reproduction of the fresh-water polyp, *Hydra grisea*, and drew conclusions which were quite in accord with the results obtained with self-fertilized lines of beans. Her results were soon called in question, however, by the work of Hase ('09) and the criticisms of Pearson ('10), who obtained such conflicting results that a re-investigation of the subject became necessary. The results of such a renewed, independent study of variation and inheritance in *Hydra* are presented in the present paper.<sup>1</sup> In order to make clear the questions at issue and the bearing of the results presented here upon these and the general problems of genetics, it will be necessary to review briefly the work of Hanel and the criticisms raised against it, especially as the latter are complicated and not clearly valid.

Before this is undertaken, however, some explanation of the terminology of the present paper may not be out of place in view of the past confusion in genetic literature. Johannsen ('13), following Shull, has employed the word 'clone' to describe a family descended from a single individual by asexual reproduction. The name 'biotype' applies to any group of organisms which show the same hereditary constitution ('genotype'). In the statistical terminology the 'distribution' is the natural arrangement of individuals showing variation ('variates') in classes. The 'mean' is the arithmetical average of the variates. The 'standard deviation' ( $\sigma$ ) is an expression of the average extent of variation from the mean shown by the variates. The 'coefficient of variation' expresses the average percentage of variation from the mean in terms of the mean as unity. The 'coefficient of correlation' ( $r$ ) represents an arbitrary measurement of the average degree of resemblance between correlated members of two series of variates. The 'coefficient of regression' ( $R$ ) expresses the average extent to which the variations of one series follow the variations of the correlated series.

The nomenclature of the genus *Hydra* has been subject to frequent changes. The names which have been used most frequently by ex-

<sup>1</sup> This was undertaken at the suggestion of Prof. H. S. Jennings, to whom I wish to express my indebtedness for his assistance and kindly criticism during the course of my work. I wish also to thank Prof. B. E. Livingston for the use of the facilities of the Laboratory of Plant Physiology of the Johns Hopkins University during the summer of 1912.

perimental zoölogists are those of Linnaeus. Brauer ('08) applied the rule of priority, thus requiring the nomenclature of Pallas. In 1912 Bedôt criticised Brauer's revision and showed that the species, *H. viridis* L. should be retained. Since the experimental literature has employed the Linnaean nomenclature this has been used here in order to avoid the constant repetition of synonyms. The names employed are given below at the left, with the revision of Bedôt at the right.

<i>H. viridis</i> L.	<i>H. viridis</i> L.
<i>H. grisea</i> L.	<i>H. vulgaris</i> Pall.
<i>H. fusca</i> L.	<i>H. oligactis</i> Pall.

*H. polypus* A. Brauer.

### *Experiments of Hanel*

Hanel began her work in 1906 and during the two years of her experiments bred clones from 26 wild Hydras, obtaining records of nearly 7000 buds, the descendants, by asexual reproduction, of the original 26. Upon comparing these 26 'stem parents' with their immediate progeny she found that those with a large number of tentacles produced buds having, on the average, a greater number of tentacles than the corresponding buds of parents with few tentacles. From the progeny of the 'stem parents' taken singly Hanel selected polyps with a high, an intermediate, and a low number of tentacles and continued this selection for from two to seven generations. Averaging the results obtained, she found that within the single clone the mean number of tentacles of the progeny of individuals selected for four generations was slightly less in the group selected for a large number than in the group selected for a small number, whence she concluded that variations within the clone are not inherited.

### *Criticisms of Hanel's results*

In 1909 Hase, at the suggestion of Plate, studied the relation of the number of tentacles of *Hydra* to the age of the polyp and to the number of tentacles of the buds. He verified the observations of Parke ('00) and Hanel that the number of tentacles increases with the age of the polyps, finding an average increase of 2.1 tentacles in a number of polyps kept under observation for 90 days. In recording her data Hanel had taken this fact

into consideration but, believing from observations upon mass cultures that the adult number of tentacles is proportional to the number which the polyps bear when they produce their first buds, she thought herself justified in computing the adult number of tentacles for young polyps which had produced a single bud. Hase concluded that the increase is not regular and that there is no true adult number of tentacles for any polyp.

Hase further believed that the number of tentacles which the buds have at the time of separation from their parents is not proportional to the number of tentacles which the parents bear at that time. He gave data to show that while the number of tentacles of the parents is increasing with age, that of the successive offspring of these parents remains constant and does not follow the increase shown by the parents. He also cut a number of polyps into two or three pieces and found that they did not regenerate the same number of tentacles that they had borne originally. From this he concluded:

Die verschiedene Tentakelzahl ist eine reine Somation und besitzt keinerlei Erblichkeitswert. Als eine reine Linie (im Sinne von Johannsen) kann man daher die direkten ungeschlechtlichen Nachkommen einer Hydra nicht bezeichnen. Es ist daher auch nicht möglich charakteristische Typen zu isolieren und sie erblich konstant zu erhalten. Aus gleichem Grund kann man auch keine reinen Linien (im vorigen Sinne) aus einer Hydrapopulation durch Selektion sortieren.

This conclusion is far from justified, however, by the evidence which Hase presents. Records of two parent Hydras are given, which increased slowly in their number of tentacles from five to nine, together with records of all the progeny of each. If the two groups are averaged, as has been done in table 1, it is evident that the buds produced by these two parents after they had acquired a large number of tentacles have a higher average than those produced when the parents had few tentacles. Yet from these data, Hase concludes, "Wir sehen, zuerst, ein scheinbares Mitgehen der Tentakelzahl der Knospen mit der Mutter, aber bald tritt ein *völliger* Rückschlag ein." As I shall show later, this is not true for *H. viridis*, as it is probably not for *H. fusca*: the regression is only partial.

The experiment upon regeneration likewise fails to support the conclusion which Hase draws from it, since it shows only that the number of tentacles is not regenerated at its full value but fails to disprove that the number regenerated is not proportional to the original number. I have reviewed the experiments of Hase in some detail because they have been generally quoted as proving that the number of tentacles of *Hydra* is not an hereditary character, whereas, at best, they but serve to cast some doubt upon Hanel's conclusions.

TABLE 1

*Increase in the average number of tentacles of buds produced while their parents bore the numbers increasing from 5 to 9 (modified from Hase)*

NUMBER OF TENTACLES BORNE BY PARENTS	MEAN NUMBER OF TENTACLES OF BUDS PRODUCED WHILE THE PARENTS BORE THE NUMBER OPPOSITE
5	5.60
6	5.63
7	6.33
8	5.80
9	6.32

A much more serious criticism of Hanel's work, and of 'pure line' work in general, was made by Pearson during the following year. He subjected Hanel's data to a more thorough analysis than she herself had done and held that they by no means justified the conclusions drawn. Concerning the evidence for the existence of strains, diverse with respect to tentacle number, he says:

Hanel begins with a very careful investigation of the growth and environmental changes in the character selected, the number of tentacles of *Hydra grisea*. There is a general agreement with Parke's results that the number of tentacles changes with age, size, food and place of culture. Differences in these factors can produce very considerable differences in individuals and differences in the averages of differentially treated groups which can amount to as much as 0.5 to 0.8 of a tentacle. These are precisely the order of the average hereditary differences. Thus:

No. of individuals	PARENT		OFFSPRING	
		No. of tentacles	No. of individuals	No. of tentacles
9		6	364	6.943
9		7	310	7.296
4		8	166	7.344
4		9	125	7.383

It will be at once recognized that the differences here are rather less than many of the environmental differences and that there is no security that these 26 foundation Hydras are really represented by differentiated hereditary numbers of tentacles. Yet this table, as it stands, embraces Hanel's proof that the number of tentacles is an hereditary character in the 'pure line.' What evidence is there that any one of the numbers of tentacles attached to those 26 parents is really constitutional and not environmental?

When we consider that Hanel's experiments extended over a period of two years, that the different 'stem parents' were collected and bred at different seasons, and that no data are given by which it is possible to judge which of the races were kept under a uniform environment this criticism greatly reduces the value of her evidence for the existence of diverse genotypes. The second point of Pearson's criticism deals with the inheritance of the number of tentacles within the clone. The data were subjected to statistical analysis by Miss Elderton and the correlation between relatives was determined for different degrees of relationship, within the population composed of the 26 clones. A comparison of the correlations showed that there is a greater resemblance between siblings and between parents and their offspring than there is between 'uncle and nephew' or between grandparents and grandchildren. This, as Pearson points out, can not be due merely to the inclusion of diverse races in the tables of correlation but indicates that there is an inheritance of variations within the clone. From this evidence Pearson concludes that Hanel's records show, not what she believed them to show, but just the reverse: that regression is only partial, both in the population and in the clone; that variations are inherited equally in both population and clone.

*Analysis of Hanel's data*

Since this method of treating the data does not deal with individual clones it seemed advisable to carry the analysis somewhat farther. I have computed the mean number of tentacles of all individuals in each of Hanel's clones and the correlation between parent and offspring for each.<sup>2</sup> These are given in tables 2 and 3. The mean numbers of tentacles of the clones

TABLE 2

*The mean number of tentacles of Hanel's clones arranged in the order of magnitude*

CLONE NO.	MEAN NO. OF TENTACLES	$\sigma$	NO. OF TENTACLES OF STEM PARENT
14.....	6.428±0.040	0.771	7
13.....	6.438±0.020	0.475	7
24.....	6.670±0.058	0.826	6
8.....	6.677±0.049	0.816	7
5.....	6.805±0.029	0.734	6
2a.....	6.925±0.054	0.692	6
1.....	6.926±0.041	0.750	6
11.....	7.010±0.031	0.786	6
12.....	7.026±0.024	0.780	6
20.....	7.170±0.031	0.721	7
2b.....	7.184±0.032	0.658	5
10.....	7.230±0.034	0.798	8
7.....	7.240±0.023	0.906	7
26.....	7.264±0.041	0.902	9
16.....	7.287±0.056	0.962	12
19.....	7.319±0.032	0.784	5
18.....	7.325±0.029	0.786	7
23.....	7.361±0.050	0.850	5
9.....	7.367±0.046	1.005	8
6.....	7.384±0.025	0.914	9
3.....	7.393±0.023	0.763	8
15.....	7.400±0.040	0.938	8
22.....	7.430±0.043	0.934	7
17.....	7.456±0.049	1.197	10
4.....	7.500±0.039	0.862	7
21.....	7.641±0.047	0.835	6
25.....	7.712±0.044	0.942	7

<sup>2</sup> Hanel records the variations in her clones in percentages; the tables contain many errors, the sum of the percentages included in one array ranging from 85 to 140 per cent. This leads to error in the means and correlations computed from the tables but these probably average out in the numerous constants determined.

form an almost unbroken series from the smallest to the largest, and, lacking evidence upon the environmental conditions, give little indication of the existence of diverse races. Further analysis indicates that the clones tended to become more alike after they had been kept under cultivation for some time. The average number of tentacles of the offspring of each of the 26 'stem parents' and the average of all their later descendants were computed and are given in table 4. The descendants of parents with extreme variations tend to revert to the mean.

The coefficients of correlation between parent and offspring within the clones (table 3) are extremely variable, but the

TABLE 3  
*Correlation between parent and progeny within each of Hanel's clones*

CLONE NO.	CORRELATION	CLONE NO.	CORRELATION
1.....	0.170±0.054	14	0.057±0.050
2a.....	0.336±0.067	15	0.323±0.040
2b.....	0.469±0.040	16	0.050±0.066
3.....	0.010±0.029	17	-0.073±0.040
4.....	-0.009±0.040	18	-0.060±0.037
5.....	0.234±0.037	19	0.072±0.041
6.....	0.142±0.026	20	-0.087±0.041
7.....	0.036±0.028	21	0.708±0.030
8.....	0.068±0.066	22	0.007±0.047
9.....	0.006±0.047	23	0.178±0.059
10.....	0.009±0.042	24	0.000±0.067
11.....	0.040±0.037	25	0.128±0.046
12.....	0.031±0.030	26	0.104±0.047
13.....	0.048±0.040	Mean of all.....	0.101

TABLE 4  
*Regression in the first and later generations of the descendants of Hanel's 26 'stem parents'*

"STEM PARENTS"; NO. OF TENTACLES	FIRST GENERATION; MEAN NO. OF TENTACLES	ALL DESCENDANTS; MEAN NO. OF TENTACLES
6	6.943	7.086
7	7.296	7.102
8	7.344	7.347
9	7.383	7.347

majority (75 per cent) are positive and the evidence favors the belief in an inheritance of variations within the clone. The great irregularity of the results, however, makes it impossible to draw from them any law of inheritance within the clone or even to be sure that there is any constant tendency toward the inheritance of variations.

### *Unsettled questions*

Thus the evidence from previous work bearing upon heredity in *Hydra* must be looked upon as inclusive and the chief problems of heredity and variation as still unsettled. Hanel maintains the existence of diverse races of *Hydra* which are distinct in their hereditary constitution. This is denied by both Hase and Pearson but upon different grounds. Hanel holds that, within a population, variations are inherited and that selection is effective in isolating the diverse races, within which there is no inheritance of variations. Hase denies that variations in the character studied are inherited at all and asserts that such variations are purely somatic. Pearson attempts to show that the variations are inherited both within the population and the clone. Analysis of Hanel's data shows that there is usually a correlation between parent and progeny within the clone; that this may be very high in some cases, and in others, negative, varying around an average of 0.101. Experimental analysis of the cause of this correlation is lacking.

The chief questions demanding further investigation seem to be the following: Will such a variable character as the number of tentacles of *Hydra* lend itself to genetic study? Are there other characters of *Hydra* which vary less within the individual? Given a character which is comparable in different individuals, do races, hereditarily diverse with respect to this character, exist? If there are such races, in what characters do they differ and are they numerous or few? Is there inheritance of individual differences within the population, and if so, what part do diverse races play in this inheritance? Is there actually a correlation between parent and progeny within the clone?

If so, how is it produced and what is its significance? Is there an inheritance of individual differences within the clone? What is the origin of the diversities between races?

## II. VARIATION IN HYDRA

The only variable character in *Hydra* considered in detail by previous workers has been the number of tentacles. At the beginning of the present experiments I examined a number of polyps in the hope of finding other variable characters suitable for genetic study. Size, body-form, color, relative length of the tentacles, reaction to mechanical stimuli, distribution of the nettle cells, diameter of the mature nematocysts, and reaction to light were compared. Of these only size seemed at all suitable for statistical study. Variations in practically all the characters of *Hydra* appear, not only between different individuals, but in the same individual at different times. Hase has shown that the number of tentacles, alone, varies too much in the individual to form a character suitable for statistical treatment and Plate ('13) has stated the necessity for the use of the number of tentacles at some definite stage in the development of the polyps in future studies of heredity. The same individual variability appears in the size of the polyps so that the size at some definite stage of development must be used in comparative studies.

The time when the bud separates from the parent marks the occurrence of certain definite physiological processes in the bud and, if the number of tentacles of the bud is taken at this time, it serves as an index of the state of development of the bud at the time when these processes are initiated. This number may be compared, in tests for heredity, either with the number borne by the parent at the corresponding age, or with the number which it bears when the given bud is produced. It is more difficult to find a stage where the size of different polyps is comparable. Growth is very rapid for the first few days after the buds are released and it is not always possible to take measurements of the new buds, as such measurements require a great deal of time. After four or five days for *H. viridis*, when the buds have matured

and begun to produce buds in their turn, growth is much slower, although the polyps continue to increase in size until the fifteenth day or later. For comparisons of parent and offspring, I have used measurements of the size at the age of seven days, or as near this as was practicable. The figures so obtained are greatly subject to chance variation but are probably as dependable as any which can be obtained for so plastic an organism.

*Variation in the number of tentacles of *Hydra viridis**

The most frequent number of tentacles (the modal number) found in wild populations of *H. viridis* is usually 6, sometimes 7, and the normal range seems to be from 4 to 9. Table 5 shows, in percentages, the distribution of variations in *H. viridis* found by previous investigators. Both local and seasonal differences probably play a part in the production of the differences in these populations. The data shown in table 6 have been obtained

TABLE 5

*Distribution, in percentages, of variations previously recorded for *Hydra viridis**

NO. OF TENTACLES.....	4	5	6	7	8	9	10	11	12	13
Rand ('99).....		2.5	42.0	40.0	12.0	2.0				
Hathaway ('99).....		11.5	50.0	35.0	3.0					
Parke ('00) I.....		32.3	48.4	12.9	4.8	1.6				
II.....		4.8	30.6	44.5	16.4	2.9	0.6			
III.....		22.8	46.5	20.9	7.4	1.4	0.9			
Reese ('09).....	?	?	?	24.0	54.0	15.0	?	?	?	?
Hase ('09).....	2.0	4.0	7.0	28.0	27.0	25.0	7.0	1.0	0.5	0.5

TABLE 6

*Distribution, in percentages, of variations in a population of *Hydra viridis* from Baltimore*

	NO. OF TENTACLES								MEAN NO. OF TENTACLES	$\sigma$	NO. OF POLYPS
	3	4	5	6	7	8	9	10			
Oct., 1911..	0.6	0.6	25.0	51.5	18.0	3.7	0.6	6.988±0.044	0.8404	167	
Oct., 1913..	0.1	0.4	5.5	30.9	50.9	11.3	0.9	6.696±0.015	0.7332	1000	
Apr., 1914..	0.7	0.7	11.4	41.4	37.9	7.1	0.7	7.393±0.050	0.8827	140	

from a population in a very small stagnant pond in the neighborhood of Baltimore. Very extensive seasonal changes are evident from the mean of the population at different times.

Numbers of tentacles less than 5 or greater than 9 probably can not be considered normal for *H. viridis* from this neighborhood. Buds formed with less than four tentacles are usually small and do not extend their tentacles. They either produce more tentacles quickly, or die. The only individuals with ten tentacles which I have found were small-bodied, dark in color, and produced buds very slowly. In *H. grisea* a large number of tentacles seems to present a rather unstable condition. Individuals with more than eight tentacles in my cultures have shown a tendency to divide lengthwise and thus reduce the number of tentacles to the mode. I have observed longitudinal division in only one specimen of *H. viridis*, which bore six tentacles at the beginning of division and gave rise to two polyps, each with six tentacles.

As individual polyps grow older they form additional tentacles, a condition first observed by Marshall ('82) in *H. viridis*. Hase has demonstrated a like condition in *H. fusca* and Hanel in *H. grisea*. Hanel has found that the addition of tentacles occurs even in polyps which are starving, although hunger tends to inhibit the process, as is clear from table 7. Parke found that the number of tentacles might be reduced and Hanel's table shows that this reduction is favored by starvation. My own

TABLE 7

*Changes in the number of tentacles of 129 specimens of Hydra grisea during one month's cultivation; after Hanel*

	WITH FOOD	WITHOUT FOOD
Number of tentacles added.....	45	19
Number of polyps showing change.....	29	14
Number of tentacles absorbed.....	2	8
Number of polyps showing change.....	2	6
Number of polyps unchanged.....	31	47
Total number of polyps.....	62	67
Mean increase per individual.....	0.69	0.16

data verify these results for *H. viridis*. Figure 1 shows the mean number of tentacles of about 30 polyps from two different clones at successive intervals of two days.

Besides the specific tendency to change the number of tentacles with advancing age, there are environmental factors which may produce variation in the number of tentacles of *Hydra*. Hanel's data, table 7, show that starvation partially inhibits

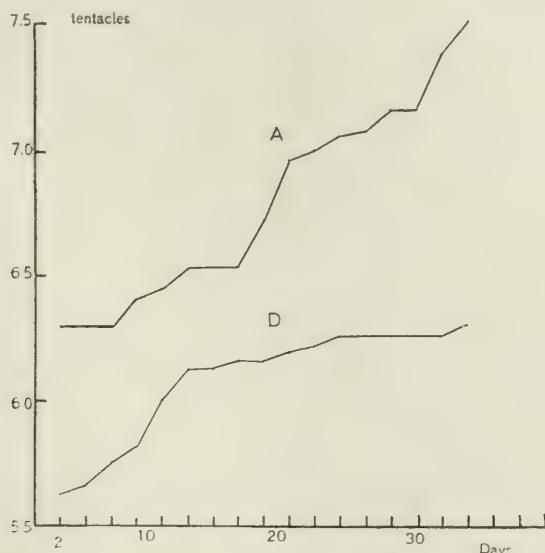


Fig. 1 Increase with advancing age in the average number of tentacles of polyps from two clones, *A* and *D*. The averages are based upon about 30 polyps from each clone. Clone *A* shows an average daily increase of 3.6 per cent; clone *D* an increase of 2.0 per cent.

the addition of tentacles and causes some to be absorbed. Parke has shown that other unfavorable conditions, such as stagnant water, have a like effect. Injury and regeneration of the oral end also leads to a reduction in the number of tentacles (Rand '99). These factors produce variation in adult *Hydras*, but further, as will be shown, they have a like effect upon the buds produced by these adults.

*Conditions producing like variations in parents and offspring*

*Age.* Corresponding to the increase in the number of tentacles with the age of the parents, the buds produced by old polyps have, on the average, more tentacles than those produced by young ones, that is, there is an increase in the number of

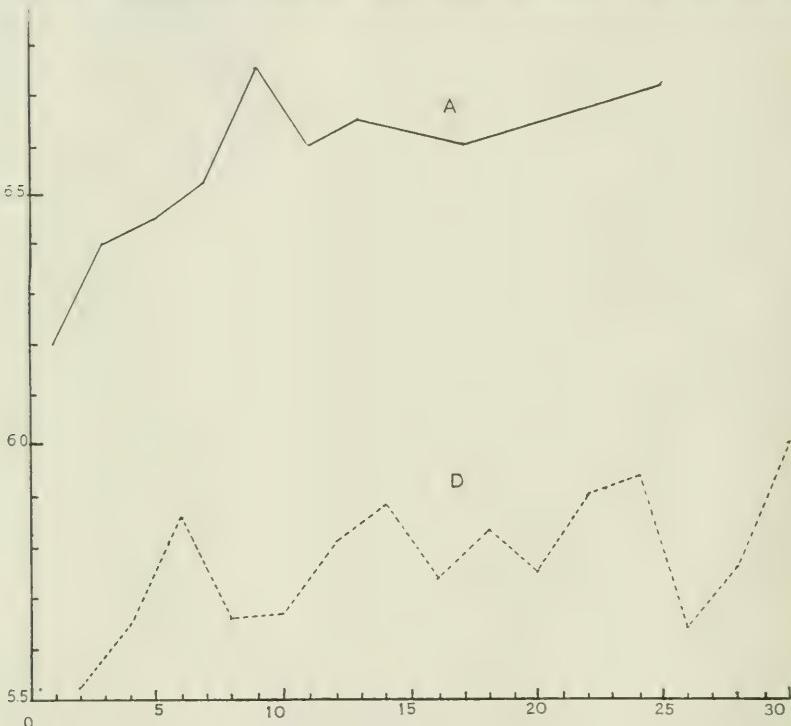


Fig. 2 Increase in the average number of tentacles of successive buds produced by the same parents. The ordinates represent the mean number of tentacles of the successive buds. The abscissae represent for Clone A the successive buds produced by 70 parents, for Clone D the buds produced on successive days after the 40 parents which furnished the basis of the curve began to produce buds.

tentacles of successive buds with the increasing age of the parent. Figure 2 shows this condition clearly. Data from two clones are included in this figure; for clone A the average number of tentacles of the first, third, fifth, seventh, etc., buds are given in the graph; for clone D the averages of all buds produced during suc-

cessive intervals of two days furnish the basis of the graph. The general trend of both lines is upward, although more markedly so in *A* than in *D*. The same relation for the two clones was found for the increase in the number of tentacles of the parents (fig. 1). The increase in the parental number of tentacles with age is due to some internal factor. Its relation to the increase in the number of tentacles of successive buds will be considered later. Besides this internal factor, there are various environmental agents which modify the number of tentacles of parent and bud in the same direction, and which must be taken into account in any study of inheritance.

*Effects of starvation.* One hundred polyps from a mass culture of a single clone (clone *A*) were divided at random into two groups of 50 each. One group was fed every second day with small crustacea, the other every sixth day. Both were placed in clean dishes of fresh water every second day, and each polyp was kept in a separate dish. The result of this method was that one group received about three times as much food as the other. The first result of the partial starvation of the six-day group was a reduction in the rate of budding. The well fed group produced an average of 0.203 buds per day for each polyp; the starved group produced only 0.074 buds per day, which gives a ratio of almost three to one, corresponding to the amount of food given. There was also a difference in the average number of tentacles of the buds produced by the two groups, the offspring of well fed parents having a slightly higher average than the others. This is shown in table 8. The difference here is small and may easily have been due to chance. The experiment was

TABLE 8

*Effects of starvation of parents upon the mean number of tentacles of their buds; all buds recorded within 24 hours after their release*

	PARENTS		BUDS	
	Mean no. of tentacles	No. of polyps	Mean no. of tentacles	No. of polyps
Fed.....	7.18±0.06	50	6.32±0.04	153
Starved.....	7.18±0.07	50	6.24±0.06	61

TABLE 9

*Effects of starvation of parents upon the number of tentacles of their buds; second experiment*

	PARENTS			BUDS		
	Mean no. of tentacles	No. of polyps	Tentacles added by all parents	Mean no. of tentacles	No. of polyps	Difference
Clone A						
Fed.....	6.84±0.05	25	0	6.59±0.03	127	
Starved.....	6.80±0.05	25	0	6.37±0.09	44	0.22±0.09
Clone D						
Fed.....	5.80±0.06	25	1	5.76±0.03	195	
Starved.....	5.92±0.06	25	0	5.20±0.06	44	0.56±0.07

repeated with polyps from two clones and results were obtained which made it certain that starvation of the parent leads to a reduction of the number of tentacles of its progeny. The results of this experiment are shown in table 9.

The parents recorded in this experiment were old polyps taken from mass cultures. Their mean number of tentacles already exceeded the means of the clones from which they came, so that any considerable increase in their numbers of tentacles during the brief time of the experiment was not to be expected. Hanel's data, however, prove that starvation tends to inhibit the addition of tentacles in parents and this experiment shows that the buds of starved parents have fewer tentacles than those of normal ones. This condition, occurring among a large number of polyps subjected to unequal and fluctuating environmental conditions would undoubtedly lead to a slight degree of resemblance between parent and progeny, even if there is no true inheritance of parental variations.

*Effect of injury.* Twenty mature Hydras were cut in two transversely and kept until both halves had regenerated. Each half was kept in a separate dish and the buds which it produced after regeneration was complete were recorded. Altogether, 287 buds were obtained from the 40 freshly regenerated polyps. The mean number of tentacles of the clone from which the original 20 were derived was, at this time,  $6.91\pm 0.03$ . The mean

number of tentacles of the original 20, the oral ends, was  $7.05 \pm 0.09$ . The mean number regenerated by the aboral ends was  $6.42 \pm 0.09$ . The regenerated oral ends produced 146 buds, the aboral ends, 132 buds within 28 days after the operations. The mean number of tentacles of the buds from the oral ends was  $6.88 \pm 0.03$ ; that of the buds from the aboral ends was  $6.59 \pm 0.04$ ; the difference here is  $0.29 \pm 0.004$  in favor of the offspring of the polyps which were required to carry out the lesser regenerative processes. Clearly, the same factors which cause a reduction in the number of tentacles in the parents (the call for an expenditure of energy in regeneration) cause also a reduction in the number of tentacles of their offspring.

In order to test the length of time during which the after-effects of injury to the parents influence the number of tentacles of the buds, the mean number of tentacles of all first, all second, etc., buds produced after regeneration by the oral and aboral ends was computed. This is shown in figure 3. After the ninth bud the graphs are based upon too small numbers to be significant. It is clear from this figure that immediately after regeneration the buds produced have few tentacles and that the number of tentacles of successive buds increases rapidly until, after five or six have been formed, the normal condition is regained.

These experiments show that certain environmental agents, acting upon *Hydra*, tend to produce variation in a given direction in both parent and offspring, either, as in the first case, by acting simultaneously upon both parent and bud, or, as in the second case, by affecting the bud only indirectly through a reduction in the vitality of the parent. Although only two such agents have been studied experimentally it is probable that many others have a like effect. Parke has shown that the bacterial conditions leading to depression in his cultures brought about a reduction in the number of tentacles of the polyps. I have found that polyps which have just recovered from depression tend to produce one or more very minute buds with few tentacles. Polyps kept in water of high salt content become small and dark-colored and produce small dark-colored buds.

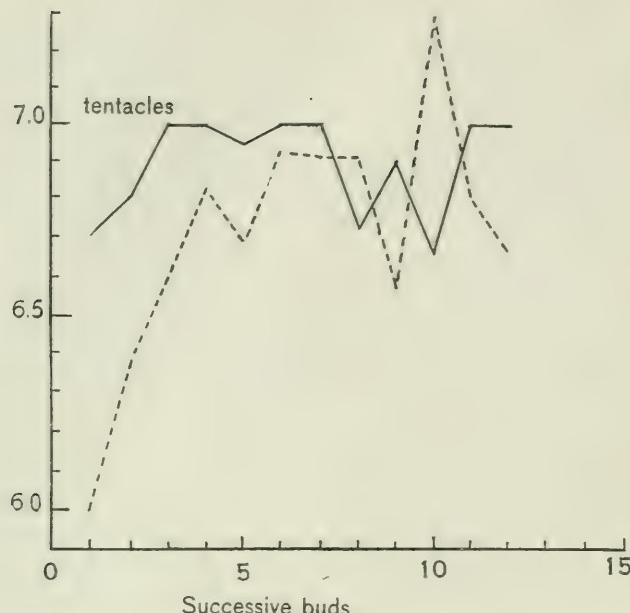


Fig. 3 The mean number of tentacles of the successive buds produced by polyps after regeneration; (—) buds produced by regenerated oral ends; (---) buds produced by regenerated aboral ends; all polyps were taken from the same clone.

Temperatures much above or below the optimum have an effect like that of high salt content. The results of the action of these agents resemble the cases of temporary parallel induction found in parthenogenetic reproduction (Agar '13) but the mechanism of the action is probably much simpler since we are dealing here only with a congenital reduction in vigor.

### III. EXISTENCE OF DIVERSE RACES

In addition to these variations resulting from environmental action, are there differences between the individuals of a population due to some internal, hereditary factors? This question has been tested by breeding a number of clones of *H. viridis* under similar cultural conditions.

### *Experimental methods*

Hydra is easily cultivated in the laboratory, provided that a constant supply of food is available. The polyps used in the experiments were collected from various sources, usually from ponds containing Nitella or Elodea. In the greater part of the work each polyp was kept separately in a small Stender dish with about 5 cc. of water. It was found necessary to wash these dishes in boiling water every second day in order to keep down bacterial growths and maintain the polyps in good health. A small species of Cyclops was used exclusively as food. Attempts to breed them in sufficient numbers in the laboratory failed and all food material was obtained by towing with a plankton net in an artificial pond. The supply so obtained was further concentrated so that from one to two hundred Cyclops were given to each Hydra every second day. (A healthy specimen of *H. viridis* will eat ten or more Cyclops daily and the large number supplied allowed each polyp to capture as many as it could eat). At first, a growing stem of Elodea was kept in the culture with each Hydra but as this was found to be unnecessary and a possible source of contamination it was discontinued after the first experiment.

All the cultures were kept at approximately the same level upon a broad table-top where they were exposed to uniform conditions of light and temperature. The cultures of various clones were divided into small groups and the order in which these were fed and arranged upon the table was varied from day to day. While conditions may have varied in the different individual cultures, there seems to be no reason for believing that such differences did not average out in each of the clones, considered as a whole.

Besides the individual cultures a number of mass cultures of different clones were kept under similar conditions. Numbers of polyps were placed in 6-inch battery jars filled with water from the food pond. The water in the cultures was renewed weekly, and where two clones were being bred for comparison the water from the culture jars containing the two clones was interchanged frequently.

The number of tentacles of the polyps was counted under the Zeiss binocular, and in the case of the polyps in individual cultures the number of tentacles of the buds was recorded within 48 hours of the time when they were released from the parents. The numbers of tentacles of the parents were recorded also at the time when each bud was produced. Measurements of size were made in the following way: The polyps, contracted, were placed on a grooved slide under a compound microscope. When they expanded until the length was about four times the diameter, an outline of the body was made with a camera lucida. The area of this outline was measured with a planimeter and from this, treated as the area of the longitudinal section of an ellipsoid, the volume was computed. This method is subject to about 25 per cent error, but is much more accurate than measurements of a single diameter. The constants given in the following pages were computed

by the simple statistical formulae devised by Pearson and others (Davenport '04; Yule '12). In the computation of the standard deviations for size Sheppard's correction was *not* used. All calculations were made with the aid of the 'Brunsviga' computing machine, and later repeated *de novo*.

#### *Detailed examination of two clones*

On June 10, 1912, twenty-five specimens of *Hydra viridis*, collected from a limited area in a small undrained pond, were isolated in individual culture dishes. They began to multiply rapidly under the favorable conditions of the cultures. The new-formed buds were each placed in a separate dish, labeled, and the number of their tentacles recorded within 48 hours after they separated from the parents. After a few days it was found impossible to care for all the descendants of the stem mothers and it seemed best to discard the majority of the clones. Two of them, *A* and *D*, which seemed somewhat diverse were retained. It soon became necessary again to reduce the number of cultures, as not more than 300 animals could be cared for at one time. An equal number of each of the two clones was therefore selected for high, intermediate, and low numbers of tentacles, and the cultures were so arranged as to give not less than ten progeny from as many parents as possible. Since the selections were approximately equal in both directions from the means of the clones, the average number of tentacles of the two may be used for comparison, although the coefficients of variation may be affected and complications are introduced in the analysis of the data for the problem of inheritance within the clone.

The breeding was continued until the latter part of August, when the first experiment was brought to a close, and the polyps on hand were placed in mass cultures and left for two weeks. At the end of this time a single polyp was taken from a mass culture of each of the clones and used as the starting point of a subordinate clone (*A'* and *D'*) bred in individual cultures as in the first experiment, except that no *Elodea* was included in the cultures.

*Differences in number of tentacles and size.* The total number of pedigree descendants of 'stem mother' *A* obtained before

September was 1353, of 'stem mother'  $D$ , 1395. The distribution of the variations in the number of tentacles of the two clones is given in table 10. The means and standard deviations computed from these figures are:

	<i>Mean</i>	$\sigma$	
Clone $A$ .....	$6.463 \pm 0.013$	0.7371	Tentacles
Clone $D$ .....	$5.739 \pm 0.011$	0.6086	Tentacles
Difference ( $A - D$ )	$0.724 \pm 0.017$		Tentacles

The difference between the average numbers of tentacles borne by polyps from the two clones is here many times its probable

TABLE 10

*The distribution of variations in the number of tentacles of the descendants of 'stem mothers'  $A$  and  $D$*

NUMBER OF TENTACLES .....	4	5	6	7	8	9
Number of polyps bearing each number of tentacles						
Clone $A$ .....	9	93	590	589	69	3
Clone $D$ .....	30	394	883	86	2	0
Percentage of all polyps bearing each number						
Clone $A$ .....	0.6	6.9	43.6	43.5	5.2	0.2
Clone $D$ .....	2.2	28.2	63.3	6.2	0.1	

error and proves that the two clones differed either in their hereditary constitution or in the environment to which they were subjected. The conditions of the experiment seem to rule out the latter possibility and further evidence against an environmental cause of the difference was given by further experiments with the clones. The second part of the experiment, beginning with the establishment of the two subordinate clones  $A'$  and  $D'$ , from single individuals taken out of mass cultures gave 204 pedigree descendants from stem mother  $A'$  and 153 descendants of stem mother  $D'$ . The number of tentacles of these polyps is shown in table 11. The constants for the distributions given are:

	<i>Mean number of tentacles</i>	$\sigma$
Clone A'.....	6.907 $\pm$ 0.026	0.557
Clone D'.....	5.844 $\pm$ 0.029	0.537
Difference (A' - D').....	1.063 $\pm$ 0.039	

After two weeks in mass culture the difference between the two clones persisted and was, indeed, increased by 35 per cent. The significance of this increase will be discussed later.

TABLE II  
*Variations in clones A' and D'*

NUMBER OF TENTACLES.....	4	5	6	7	8
Number of polyps					
Clone A'.....		3	33	148	20
Clone D'.....	2	29	114	7	1

Similar results were obtained from populations of the two clones grown in mass culture. The results from five pairs of mass cultures will be considered.

Two battery jars were filled with pond water; to each a few Elodea stalks free from foreign Hydras were added; and each was inoculated with 50 polyps, the one from Clone A, the other from Clone D. Cyclops were added every week for three weeks. At the end of a month 100 polyps were taken from each culture and the numbers of their tentacles were recorded. The variations were distributed as shown in table 12, I. The constants obtained from this are:

	<i>Mean number of tentacles</i>	$\sigma$
Clone A.....	6.56 $\pm$ 0.056	0.8284
Clone D.....	5.86 $\pm$ 0.033	0.4903
Difference (A - D).....	0.70 $\pm$ 0.060	

Five weeks later samples were again taken from these cultures. Their variations are shown in table 12, II. The constants for these groups are:

	<i>Mean number of tentacles</i>	$\sigma$
Clone A.....	6.797 $\pm$ 0.042	0.5038
Clone D.....	5.457 $\pm$ 0.045	0.5539
Difference (A - D).....	1.340 $\pm$ 0.061	

TABLE 12  
*Distribution of variations in samples taken from mass cultures*

NUMBER OF TENTACLES.....	4	5	6	7	8
I After three weeks with plentiful food					
Clone A.....	1	9	33	47	10
Clone D.....		20	74	6	
II After five weeks more under same conditions					
Clone A.....		1	13	48	2
Clone D.....	2	34	34		
III After one month with little food					
Clone A.....		1	17	28	4
Clone D.....		26	24		
IV After three months in mass cultures					
Clone A.....		1	12	33	4
Clone D.....		25	24	1	

Polyps from mass cultures to which no food had been added for one month showed the distribution of table 12, III. The constants for these groups are:

	Mean number of tentacles	$\sigma$
Clone A.....	.6.70±0.06	0.6403
Clone D.....	.5.48±0.05	0.4995
Difference ( $A - D$ ).....	.1.22±0.08	.

The samples recorded in table 12, IV, were taken from mass cultures three months old. The constants for these groups are:

	Mean number of tentacles	$\sigma$
Clone A.....	.6.80±0.06	0.6000
Clone D.....	.5.52±0.05	0.5380
Difference ( $A - D$ ).....	.1.28±0.08	.

The Hydras in one pair of cultures were given very little food for three months and the water in the cultures was changed very rarely. As a result of these unfavorable conditions the polyps in both cultures were very much reduced in size and in number of tentacles. Clone A was most affected but the difference between the clones persisted even here, as the following constants for these populations show:

	<i>Mean number of tentacles</i>	$\sigma$
Clone A.....	5.519 $\pm$ 0.035	0.5325
Clone D.....	5.346 $\pm$ 0.033	0.4970
Difference ( $A - D$ ).....	0.173 $\pm$ 0.048	

The second character studied in these clones was the size of the polyps when about one week old. The measurements include 184 individuals of Clone A and 182 of Clone D. The

TABLE 13  
*Distribution of variations in the size of clones A and D*

	CUBIC MILLIMETERS												
	0.00	0.16	0.32	0.48	0.64	0.80	0.96	1.12	1.28	1.44	1.60	1.76	1.92
Clone A.....	10	25	22	28	28	26	13	11	8	4	1	8	
Clone D.....	24	78	55	17	7	1							

distribution of the variations is shown in table 13. The mean sizes of the two clones are:

	<i>Mean size</i> cu. mm.	$\sigma$
Clone A.....	0.869 $\pm$ 0.021	0.4288
Clone D.....	0.322 $\pm$ 0.022	0.1504
Difference ( $A - D$ ).....	0.547 $\pm$ 0.023	

The mean age at the time of measurement for members of the two clones was:

Clone A 7.1 days..... Clone D 7.8 days ..

The difference in size is even more pronounced than the difference in the number of tentacles: individuals from Clone A were, on the average, more than twice as large as those from Clone D.<sup>3</sup> The difference in the appearance of the polyps is shown in figure 4. The numbers measured are relatively small but they extend

<sup>3</sup> An attempt was made to determine whether the difference in size was due to a difference in the size of the individual cells, in accordance with the theory of the relation between cell and body size advanced by Popoff ('08). Because of the great mobility of Hydra, accurate measurements could be obtained only for the mature nematocysts. In this character the two clones were identical and if it furnishes an index to the size of other cells the difference between the clones was due to a difference in the number and not the size of the body cells.

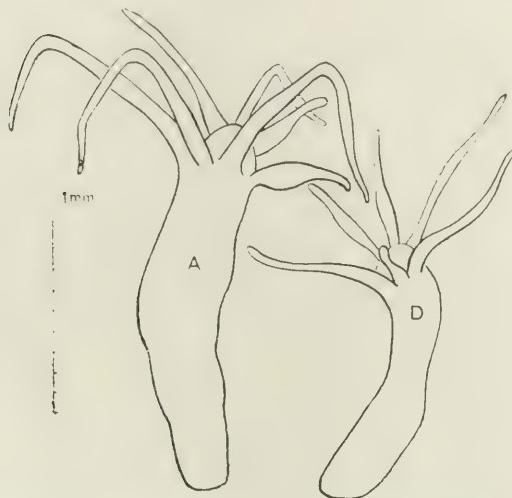


Fig. 4 Diagram of the size relations of Clones *A* and *D*. From camera drawings of polyps of mean size.

over more than half the period of cultivation so that environmental differences should have averaged out. The polyps measured were selected because of their relationships, so that the personal factor, an unconscious selection of extremes, can have played no part in producing the difference.

All the differences recorded are between groups which had been kept under environmental conditions as nearly uniform as possible. The polyps in individual cultures were fed in irregular order in order to insure a uniform distribution of food. In the mass cultures the parallel series of the two clones were given uniform treatment and an interchange of water in the parallel cultures was made frequently. The evidence seems to prove conclusively that some internal, hereditary factor caused the differences between the clones.

This conclusion is still further verified by an analysis of the data from the individual cultures. In order to test the constancy of the difference, the time during which the clones were cultivated was divided arbitrarily into intervals of five days and the mean number of tentacles of all buds produced by each clone during each five-day period was computed. Table 14 and figure

TABLE 14

*Constants for the number of tentacles of the buds produced by clones A and D during successive five-day intervals from June 25 to August 23*

NO. OF PERIOD	CLONE A		CLONE D		(A - D)
	Mean no. of tentacles	$\sigma$	Mean no. of tentacles	$\sigma$	
1.....	6.530±0.099	0.6056	5.500±0.168	0.5000	1.030±0.194
2.....	6.115±0.118	0.8913	5.462±0.091	0.6919	0.653±0.149
3.....	6.223±0.046	0.7280	5.479±0.036	0.6204	0.744±0.053
4.....	6.142±0.055	0.8478	5.345±0.027	0.5679	0.796±0.061
5.....	6.233±0.055	0.7754	5.441±0.036	0.6257	0.792±0.065
6.....	6.552±0.031	0.6529	5.796±0.029	0.5532	0.756±0.042
7.....	6.795±0.030	0.6268	6.103±0.031	0.6386	0.692±0.043
8.....	6.681±0.033	0.6334	5.958±0.023	0.4542	0.723±0.040
9.....	6.439±0.036	0.6499	5.757±0.029	0.5078	0.682±0.046
10.....	6.236±0.038	0.7502	5.787±0.030	0.4980	0.449±0.042
11.....	6.396±0.043	0.5958	5.787±0.036	0.5376	0.972±0.056
12.....	6.384±0.090	0.6836	5.775±0.090	0.4875	0.609±0.128

5 show the result of this analysis. Throughout the entire experiment the difference between the clones persisted. By July 25 Clone D had increased in the average number of tentacles of its buds until it equaled the earlier average of Clone A (June 30) but this was accompanied by a corresponding increase in the average of Clone A. The figure shows that the difference remained fairly constant in spite of wide fluctuations in the absolute values of the means of the clones.

*Other differences between the clones.* There is a marked difference between the clones with respect to the age at which reproductive maturity is reached, the age at which the parents produce their first bud. This is shown in the following tabulation:

	Mean age of parents when first bud was produced	$\sigma$	No. of parents
Clone A.....	4.806±0.103 days	2.422	248
Clone D.....	3.748±0.074 days	1.755	253
Difference (A - D)	1.058±0.127 days		

The rate of reproduction of the clones differed slightly, that of Clone A being somewhat greater than that of Clone D. The difference, based upon 33 parents of each clone which produced more than 10 buds is 0.0542 buds per day. This difference is

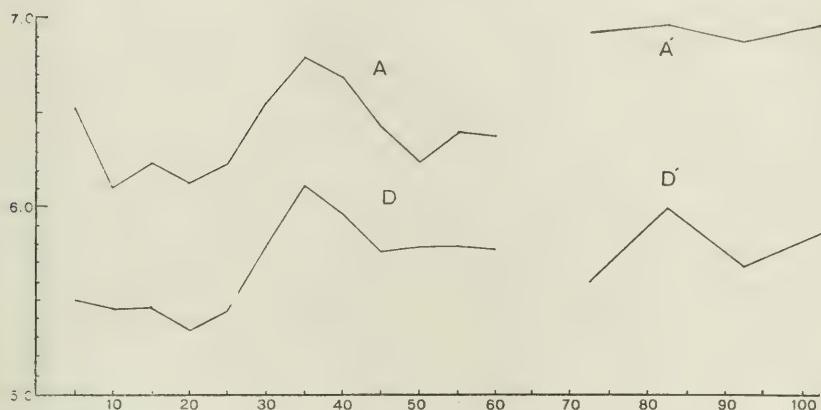


Fig. 5 The mean number of tentacles of the buds produced by Clones *A* and *D* during successive intervals of five days. The mean number of tentacles of Clones *A'* and *D'* are included at the right as these form a natural continuation of the earlier experiment.

not greater than its probable error and is probably not significant. The equality in reproductive rate is shown by the fact that while no attempt was made to obtain equal numbers in the two clones during the experiment, the numbers bred were, after two months, almost equal (1353 and 1395).

Other differences between the clones which can not be expressed quantitatively were apparent in the living animals. Clone *D* was, as a rule, somewhat darker in color than Clone *A* and somewhat more resistant to unfavorable conditions. Polyps of Clone *A* showed a greater tendency to cling to the surface film and were more active than those of Clone *D*.

*Nature of the difference between Clones *A* and *D*.* The foregoing data prove that the two clones studied are distinct through the presence of some internal, hereditary factor. The 'Reaktionsnormen' of the two are different. But whether this difference is genotypic in the sense of being the result of a fixed constitution of the germ-plasm or whether it is due to a spurious heredity like that by which the green color is transmitted is not clear. The agents, other than the hereditary constitution, which might produce such clonal diversities are the direct action of the

environment, the persistent effects of former diversities of environment, variations in the commensal relations of the polyps and their zoothiorellae, parasitic infection of one clone, and differences in clonal age from the fertilized egg. The first of these is eliminated by the methods of the experiment. It has been shown that the after effects of injury and 'depression' are only temporary. Experiments attempting to produce permanent modification by extremes of temperature, chemical agents, and artificially induced 'depressions' have been unsuccessful; diversities are readily produced but they persist for only a few days.

Possible variations in the commensal relations of *H. viridis* are more difficult to control. Microscopic examination of the two clones failed to reveal any difference in the green bodies of the two clones. As a test of the influence of commensalism upon variations I partially removed the zoothiorellae from some individuals of Clone *L* (see below) by the method devised by Whitney ('06-'08). During immersion in glycerin the polyps became smaller but when restored to normal conditions they ate regularly and resumed their original size before beginning to bud. The buds, practically white in color, did not seem otherwise different from the normal members of the clone. I never succeeded in getting out all the zoothiorellae from the polyps so that not enough buds for statistical study could be obtained before the polyps resumed their normal color.

Five parasites of *Hydra* have been reported: *Amoeba hydroxena* Entz, *Balantidium hydrae* Entz, a species of *Ophryoglena* (Entz '12), *Trichodina pediculus* Ehr. (Clarke '65), and *Kerona polyporum* Ehr. The true parasitic nature of only one of these, *A. hydroxena*, has been demonstrated. *Trichodina* did not occur in any of the pedigree cultures recorded here, although it is common in wild populations. In October, 1913, an epidemic of *A. hydroxena* broke out in my cultures, destroying all of them. The symptoms of infection by this parasite are easily recognizable and there is no possibility that it occurred in the earlier cultures. *Kerona* occurs rarely in wild populations here but did not appear in my cultures; the other two parasites have

never been seen in this locality. The fungus mentioned by Rand ('99) is evidently not a parasite of *Hydra*.

Finally, there is no direct evidence bearing upon the question of the effects of the age of the clone upon its variations. It has been impossible to secure clones of known age since in this locality sexual reproduction in *H. viridis* seems to be almost completely suppressed. Hydras from wild populations have been examined at all seasons during the past three years and in this time not one with ovaries and only seven with spermares have been found. The treatment by which Whitney ('07) induced sexual reproduction was tried but without success. These facts seem to justify the conclusion that sexual reproduction plays but little part in the life history of the green polyp.

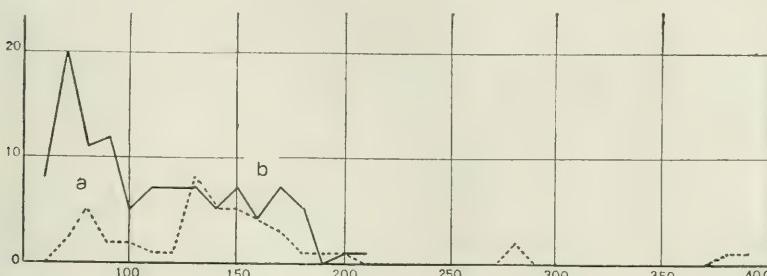


Fig. 6 The number of polyps (ordinates) which died at the ages in days recorded on the abscissae, during Hase's experiments. (—) *H. fusca*, (---) *H. grisea*.

In the development of theories of 'life cycles' in micro-organisms the phenomena of 'depression' in *Hydra* have had a prominent place. R. Hertwig, in particular, has sought to emphasize the relations of 'depression' to sexual reproduction, but the work of Frischolz ('09) seems to prove that no such relation exists. Practically all studies of depression have been carried out with polyps in mass cultures and there is no evidence that the epidemics of depression reported were not due to environmental rather than to internal factors. The ease with which depression may be induced by unfavorable environment and cured by putting the polyps in clean water makes it practically certain that depression is a pathological condition.

The only other work bearing upon the question of age is that of Hase, who studied the age of individual polyps. He found a mean age of 55.2 days for *H. fusca* and 94.8 days for *H. grisea*, with the distribution

of ages at death shown in figure 6. The irregular form of the curves and the fact that the vast majority of the polyps died at a relatively early age scarcely speaks in favor of an 'Altersschwäche.' The curves are similar enough in form to suggest that the deaths were due to two periods of unfavorable conditions (*a* and *b*) in his cultures.

At no time have I observed an epidemic of depression in the individual cultures belonging to the same clone; less than 1 per cent of the polyps have shown depression and always the closest relatives of these remained normal, a condition which is not at all in harmony with theories of clonal senescence. Further, during the four months that the clones were kept under observation, including more than 20 asexual generations, there was no significant change in the character of either clone. The reproductive rate, the first character modified by a reduction of the vitality of the polyps, was as high at the end of the experiment as at the beginning. This furnishes some evidence against the belief that the diversities are due to the clonal age but it is not conclusive.<sup>4</sup>

#### *The existence of other races*

A comparison of the standard deviations of the Clones *A* and *D* reveals the fact that clone *A* was always the more variable of the two. This difference suggested that Clone *A* was not pure and an examination of other evidence quickly confirmed this view. The variations of the clone tended to a dimodality which does not appear in any other race studied and the greater variability and dimodal form of the variation curve did not persist in Clone *A'* which was descended from a single polyp taken from Clone *A*. The records of the clone were reviewed in order to test whether the greater variability was characteristic of the race or was the result of the inclusion of diverse types as is suggested by the condition in Clone *A'*. One subordinate clone represented by the direct line of descent A1A2b1a1a2a1a (the

<sup>4</sup> Agar ('14) has shown that diverse clones produced by parthenogenetic reproduction may be produced by different eggs hatched at the same time. The differences here are clearly not due to the ages of the clones. Lang ('92) found that only one germinal layer of Hydra takes part in the formation of the buds, and this work, while unconfirmed, suggests that the differences between parthenogenesis and budding may not be so great as is generally supposed.

successive buds of alternate generations being labeled with the series of letters and numerals) showed an average number of tentacles considerably lower than that of the remaining portion of the clone. The polyp *A1A* was recorded as small, with abnormal tentacles, rapidly becoming normal. The mean number of tentacles of the fraternity from which it came was  $6.809 \pm 0.062$ , that of its immediate progeny was  $6.200 \pm 0.023$ , giving a reduction of  $0.609 \pm 0.067$  tentacles in one generation.

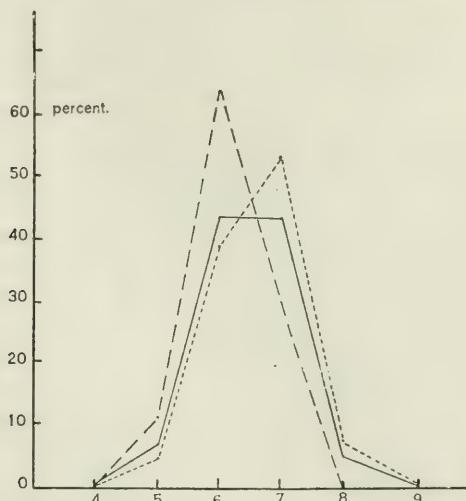


Fig. 7. The distribution of variations in the number of tentacles of Clone *A* (—) and of the subordinate clones *A1A* (---) and *A*—(*A1A*) (- - -).

The mean of all the descendants of *A1A* (437) was  $6.121 \pm 0.020$ : that of the remainder of the clone was  $6.624 \pm 0.013$  giving a difference of  $0.503 \pm 0.024$ . The distribution of variations in the two subordinate clones, separately and combined, is shown in figure 7. The dimodality of Clone *A* is clearly the result of the combining of these two groups (neither subordinate clone breaks up upon further analysis nor does any other method of dividing Clone *A* reduce its dimodality. The appearance of this diversity gives no evidence of a cumulative inheritance of variations. The change was quite sudden, resulting in a difference after one generation as great as the difference in any later gener-

ation. The individual *A1A* must be looked upon either as a mutant or as, more probably, a polyp of another clone introduced by accident into the culture.<sup>5</sup> It seems to be hereditarily different both from Clone *D* and from the remainder of Clone *A*.

Diverse races which can be recognized at a glance are extremely rare in the populations near Baltimore. Several times fifty

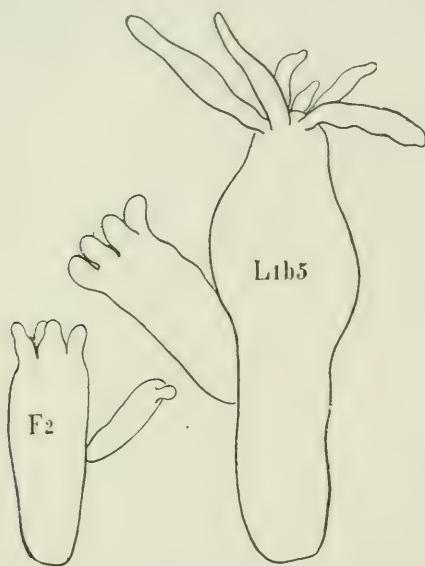


Fig. 8 Diagram of the size relations of two clones kept under similar conditions, *L* and *F*. From camera sketches of individuals of estimated average size.

or more clones have been started with polyps from different localities but only twice have distinct races appeared which were recognizable within a few generations. The first of these were Clones *A* and *D*. In August, 1913, a small polyp was found which produced very small buds. At first it seemed unhealthy but the buds increased in size and began to multiply rapidly. Unfortunately the culture could not be continued and the clone was

<sup>5</sup> The opportunity for contamination was given by the use of Elodea stalks in these cultures, as in the early part of the experiment no certain method of freeing them from foreign Hydras had been devised.

TABLE 15

*A comparison of the mean number of tentacles of clones T, L, and R*

CLONE	NO. OF TENTACLES OF STEM MOTHER	MEAN NO. OF TENTACLES OF DESCENDANTS	NO. OF DESCENDANTS
T.....	6	6.553±0.058	56
L.....	6	6.282±0.049	46
R.....	8	6.650±0.081	40
T.....		6.513±0.033	154
L.....		6.184±0.033	152

$$\text{Difference } (T - L) \quad 0.328 \pm 0.046$$

lost before many individuals had been obtained. However, the difference between this and another clone, *L*, kept under the same conditions was so marked that it seems certain that the small race represented a distinct genotype. The size relations of the polyps of the two clones is shown in figure 8, taken from camera lucida sketches of estimated average individuals.

More frequently the differences are less pronounced. Table 15 gives the constants obtained for three clones bred during July and August, 1913. Clones *T* and *L* were continued after *R* was discarded and the constants for their full numbers are given separately. They were grown in individual cultures under parallel conditions as in the first experiment and seem to be hereditarily distinct.

These are the only races which I have cultivated under exactly parallel conditions and no evidence upon the number and variety of such races is available.

*Varieties of Hydra in the earlier literature.* The extensive literature dealing with *Hydra* gives frequent suggestions of the existence of local varieties of the three generally recognized species, and one or two descriptions seems really to establish their existence. The statements concerning the size of *H. viridis* made by Baker, Trembley, Rosel, Pallas, and Kästner differ considerably but are not conclusive. Marshall ('82) described a variety of *H. viridis* from brackish water which remained distinct from the fresh water forms even after cultivation for

many generations in fresh water. The systematic literature and the studies of gonad production by Brauer, Downing Nussbaum, Weltner, and Koelitz, suggest the existence of monoecious and dioecious varieties. The experiments of Tower show two types of *H. viridis* differing in their reaction to the ultra-violet rays (?) and in the time required for regeneration.<sup>6</sup> Annandale has described a variety of *H. grisea* from India which is characterized by having a four-tentacled winter form producing gonads. Whitney has been able to establish varieties of *H. viridis* without green bodies.

### *Summary*

To sum up the present section of the work: It has been found that within a wild population of *H. viridis* there are hereditarily diverse races which differ in their number of tentacles at separation from the parent, in their size at a given age, and less certainly in other characters. The differences between such races are permanent so long as the races are kept under the same environment. The evidence favors the view that the differences are truly genotypic (with the reservation that they are possibly the result of differences in the age of the clones).

#### IV. INHERITANCE OF VARIATIONS WITHIN THE CLONE

Inheritance of variations within the clone may be tested in two ways; first, by an analysis of the resemblance between parent and progeny by statistical methods; second, by a continued selection of variates, which should change the type of the clone if the variations are inherited. As has been shown in the discussion of Hanel's data, selection of variates within the clone seems, on the average, to have no effect, yet there is usually a positive correlation of parent and progeny with respect to the selected character. Pearson has subjected Johannsen's data for the selection experiment with beans to a similar analysis and

<sup>6</sup> I have made many attempts to repeat Tower's experiments, using different types of arcs, with and without interposed glass, but have never obtained the sloughing of the ectoderm which he describes.

has shown that in these pure lines there is likewise a correlation between parent and progeny. His explanation of this seemingly anomalous condition and the criticism which has been urged against selection experiments in general where Vilmorin's method is employed is based upon the small numbers selected. Pearson ('10), discussing Hanel's results, says:

In selecting a few isolated individuals in each generation, where non-hereditary influences are so influential, we may break the influence of heredity at each step, and since such influences are equally effective with heredity, the chances are that we will do so once in every *two* selections. Only by taking large numbers of the high and large numbers of the low would it have been possible to average out the effects of environmental changes.

The only ways in which this criticism can be met are by very extensive selections involving large numbers of individuals, or by a study of variation and environment which shall make possible a differentiation between heritable and non-heritable variations, upon some other basis than that of ancestral correlation.

#### *Resemblance of close relatives within the clone*

*Number of tentacles.* In all breeding experiments the polyps have been so recorded that the number of tentacles of the buds can be compared with that borne by their parents, both at the time when the buds were produced and when the parents themselves were buds. Comparisons of both numbers must be made in order to answer two distinct questions: (1) do the offspring resemble their parents when the latter were in the same stage of development; (2) are the variations of the parents during their development transmitted?

To test the first question the correlation between the initial number of tentacles of the parents and that of their buds was computed. Since there is reason to believe that Clone *A* is impure, it was divided into the two subordinate clones mentioned earlier and the constants were computed for these. If there is a cumulative inheritance of slight variations this division should affect only the probable errors of the correlation constants. The

coefficients of correlation obtained in this way are given in table 16. Only one of these, that of Clone *D'* is more than twice its probable error, and this one is negative. The others are all too small to have any significance. The large negative correlation of Clone *D'* is the result of the inclusion of a single parent (the stem parent of the clone) which produced a large number of progeny with few tentacles and was thus heavily weighted in the computation of the coefficient.

The Clones *A*—(*A1A*) and *D* contain such large numbers both of parents and offspring that it is almost certain that the low correlation is not the result of chance, but truly expresses an almost total absence of similar variations in parent and progeny within the clone. In so far as the coefficient of correlation is a reliable test of heredity we may conclude that there is no inheritance of variations in the initial number of tentacles of *Hydra viridis*.

For Clone *D* the fraternal and grandparental correlations were likewise computed; they are given in table 17. Like the parental correlation, the grandparental is too small to have significance.

TABLE 16

*Correlation of the initial number of tentacles of parents and progeny within the clone*

	<i>r</i>	NO. OF PARENTS	NO. OF PROGENY
Clone <i>D</i> .....	0.0038±0.018	251	1395
Clone <i>A</i> —( <i>A1A</i> ).....	0.0011±0.023		859
Clone <i>A1A</i> .....	0.0342±0.032		439
Clone <i>D'</i> .....	0.2420±0.051	18	153
Clone <i>A'</i> .....	0.0310±0.047	28	204
Clone <i>T</i> .....	0.0750±0.054	51	154

TABLE 17

*Ancestral correlations, clone D*

<i>i</i>	<i>r</i>	NO. OF PARENTS	NO. OF PROGENY (PAIRS)
Parental.....	0.0038±0.018	251	1395
Grandparental.....	-0.0495±0.018	68	1307
Fraternal.....	0.0770±0.006	.	10766

The fraternal is only slightly greater and its value is doubtful. It will be considered again in the discussion of the cause of ancestral correlation within pure lines and clones.

The second question, do the parents transmit to their buds the characters which they have when the buds are formed, involves one of two concepts. Either the effects of environmental action must be transmitted or the organism must continually change in its hereditary potentialities during its development in order that its transient characters shall be inherited. Something of the latter conception seems to be implied in Pearson's doctrine of homotyposis.

TABLE 18

*Correlation between the original number of tentacles of buds and the number borne by the parents at the time when each bud was produced*

	r	NO. OF PARENTS	NO. OF PROGENY
Clone D.....	0.096±0.016	251	1395
Clone A.....	0.240±0.009	242	1353
Clone A. Grandparental....	0.229±0.013		1094

The correlation of the number of tentacles of the buds with that of the number borne by the parents when each bud was produced has been computed for Clones *D* and *A*; these are given in table 18. The high correlation of Clone *A* is obviously the result of the inclusion of the two diverse strains but a comparison of the parental and grandparental correlations within this clone shows that there is a slight positive parental correlation which is not the result of the mixture of the two types. (The parental correlation would not be higher than the grandparental if the inclusion of diverse types were the only cause of correlation). This correlation is certainly very small, and that of Clone *D* is also too small to have significance when considered alone, but the fact that these correlations are greater than the ones obtained for the initial number of tentacles indicates that there is here some factor tending to produce a resemblance between the mature parent and its buds. This is also the impression

given by the average parental correlation of 0.101 found for Hanel's clones.

What is the significance of such a small coefficient of correlation in an organism so subject to environmental influence as is *Hydra*? Pearson holds that the presence of variations due to environment would tend to obscure the real correlation between parents and offspring and hide any real inheritance which might exist. My data upon the relations of variation to environmental changes indicates that the latter may be more effective in producing a likeness between close relatives than in obscuring such a likeness. Some conditions producing like variations in parent and offspring in *Hydra* have been considered already. Any great diversities in the conditions of the cultures would result in a correlation between parent and progeny, even though there were no inheritance of the variations studied. Such conditions have been found by Agar in daphnids and plant lice and probably account for the ancestral correlations found by Warren in these forms.

The production of a correlation in *Hydra* by the action of diversities of environment may be illustrated by a consideration of the change in the mean of clones during long periods of cultivation. Figure 5 shows that the mean number of tentacles of the buds produced by Clones *A* and *D* during the first few weeks of cultivation was low; that it increased gradually during the first six weeks, and then decreased again. All the buds produced during each of these five-day intervals were correlated with one another. This gave the following results:

	<i>Coefficient of correlation</i>	<i>No. of pairs</i>
Clone <i>A</i> .....	0.0774±0.0015	95141
Clone <i>D</i> .....	0.1313±0.0014	101872

Correlation of all buds produced in each five day period with all produced in the preceding period (a time interval corresponding to a full generation) gives for Clone *A* a correlation of 0.048±0.001 which is greater than any of the parental correlations found for the initial number of tentacles.

These correlations show a resemblance between the buds produced within a limited time as great as that found between the closest relatives, although these buds were no more closely related to each other than to those produced during other five day intervals. This same effect would be visible in the correlation between parent and progeny whenever several generations are included in the same correlation table, as is necessary in the case of *Hydra*.

Such effects of environmental action seem adequate to account for all the coefficients of correlation given by Hanel's data and for the very slight positive correlations found for the initial number of tentacles in my own clones. The fact that the number of tentacles of successive buds increases with the increase in the number of tentacles of the parent accounts for the slightly larger correlation found between the number of tentacles of the parents and offspring recorded when each bud was produced. Whether the increase in the number of tentacles of the buds is an inheritance of the variations taking place in the parent with growth, or is only a temporary effect of the increased vigor of the parent, must be tested by the success or failure of an attempt to modify the character of the race by the continued selection of variates.

#### *The effects of selection within the clone*

The second method of testing inheritance, that of continued selection of variates, offers a good many practical difficulties in *Hydra* owing to the sensitiveness of the polyps to environmental changes. A specimen of *H. viridis*, collected in the late summer of 1914, was used to found a large clone. Its progeny were bred in individual cultures until 85 members of the clone were obtained. These gave a mean of  $6.141 \pm 0.058$  with the distribution shown in table 19. From this clonal population 25 polyps with seven tentacles and 25 with six or less were selected. Each was kept until it produced a bud varying from the mean in the same direction as itself. This bud was then selected and kept in the same way until it in turn produced a bud varying in the same direction, and this selection was continued for three

TABLE 19

*Distribution of variates in the clone from which the fifty parents of the selected lines were taken. The polyp from which this clone was derived bore 9 tentacles*

Number of tentacles.....	3	4	5	6	7	
Number of polyps.....	1	2	9	45	28	85
Mean.....						$6.141 \pm 0.058$

months. At the end of this time a record was kept of all the progeny of the last selected generation in each of the 50 lines. The continued freezing of the food pond made it necessary to bring the experiment to a close when an average of 12 buds had been obtained from each of the members of the last selected generation. The selection covered an average of 6.08 generations in the group selected for seven or more tentacles, and of 7.92 generations in the group selected for six or less. The average number of tentacles of all selected generations of the plus selected group was 7.008, that of all selected generations of the minus selected group was 5.560, giving an average of 1.448 tentacles as the amount of difference per generation between the selected ancestors of the two groups.

The first effect of the selection was a marked reduction in the vitality of the group selected for a small number of tentacles. The rate of budding of the group was reduced and some of the polyps showed symptoms of slight depression. Four of the buds of the last selected generation of this group, after maturing and producing from four to eight buds, went into a depression which lasted for a week or more and were revived only with difficulty. The reduction in the vigor of the minus selected group introduces a complication into the study of the effects of selection, for the transmission of reduced vitality, or of characters dependent upon reduced vitality, is not a proof of heredity, unless, indeed, the changed condition prove quite permanent.

The total number of progeny obtained from the last selected generation was 583, of which 309 were from the parents selected for a large number, and 274 from those selected for a small number of tentacles. The distribution of variations in the two

groups is shown in table 20. The offspring of the plus selected group have an average number of tentacles  $0.093 \pm 0.035$  higher than that of the minus selected group, which as an effect of selection is scarcely significant. It is about what would be expected for a strength of heredity expressed by the parental correlation of 0.01 (table 21).

Whether this effect of selection proves that the progeny inherit one one-hundredth of the variation of their parents, or is merely the effect of a temporary modification in the vigor of the selected groups may be tested by a comparison of the successive offspring of the last selected generation. Such a comparison shows that the entire difference between the two groups appeared in the first six buds produced. The average number of tentacles of successive buds in the two groups increased

TABLE 20

*Variations in the number of tentacles of the offspring of the last selected generation of polyps selected for a large and a small number of tentacles*

	4	5	6	7	8	MEAN
Selected for a large number...		7	89	204	9	$6.695 \pm 0.023$
Selected for a small number...	2	9	91	165	7	$6.605 \pm 0.026$
Difference in the direction of selection.....						$0.095 \pm 0.035$

TABLE 21

*Theoretical effect of selection of parents differing by 1.45 tentacles continued for  $F_n$  generations when the strength of inheritance is that indicated by the coefficients of correlation given*

COEFFICIENTS OF CORRELATION	0.50	0.10	0.05	0.01
Difference at successive generations.				
Parental generation.....	0.000	0.000	0.000	0.000
$F_1$	0.725	0.145	0.073	0.015
$F_2$	1.098	0.276	0.141	0.029
$F_3$	1.274	0.393	0.207	0.043
$F_4$	1.362	0.498	0.269	0.057
$F_5$	1.406	0.594	0.328	0.071
$F_6$	1.428	0.689	0.384	0.084

TABLE 22

*Averages of the first six and of all later buds produced by polyps selected for a large and of those selected for a small number of tentacles*

	AVERAGE OF FIRST SIX BUDS	AVERAGE OF ALL LATER BUDS
Parents selected for a large number of tentacles.....	6.677±0.029	6.712±0.030
Parents selected for a small number of tentacles.....	6.460±0.034	6.782±0.037
Difference in the direction of selection...	0.217±0.044	-0.070±0.047

as usual but those of the minus selected group increased more rapidly and to a greater extent than that of the plus selected one. This is shown in figure 9. The averages of the first six and of all later buds of the two groups is given in table 22. The difference in favor of the plus selected group in the first six buds is

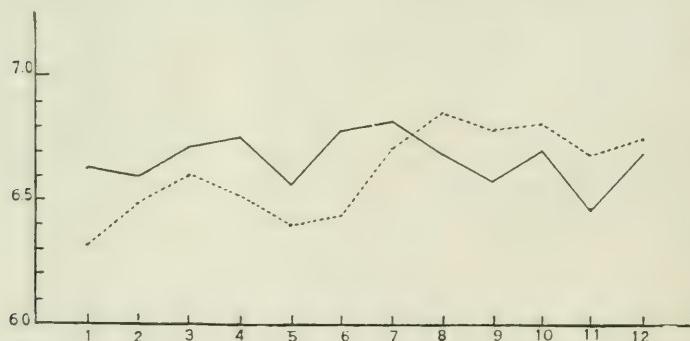


Fig. 9 The average number of tentacles of the successive buds produced by the last selected generation in the selection experiment; (—) ancestry selected for a large number of tentacles; (---) ancestry selected for a small number of tentacles.

$0.217 \pm 0.044$ . There is no significant difference between the later buds of the two groups.

Since not all parents produced the same number of buds it seemed possible that this decrease in the difference between the groups might be due to a larger percentage of the progeny of more vigorous polyps among the buds produced after the sixth, but

the data given in figure 9 shows that this is not the case. There is no difference between the averages of the seventh and of the eighth buds of the two groups and all parents produced at least eight buds.

The likeness of this result to that obtained from regenerating polyps (fig. 3) is very striking and there can be little doubt that it is due to the same cause, a reduction in the vitality of one of the groups compared. Selection of polyps with few tentacles resulted in the selection of those with low vitality and as soon as these were given time to regain their health they produced buds varying around the mean of the race. Selection produced no permanent change in the type of the clone studied.

### *Inheritance of size*

From the measurements of the size of polyps in Clones *A* and *D* the correlations in size between parents and offspring and grandparents and grandchildren within these clones were computed. These coefficients are given in table 23. In every case they are positive and the parental correlation of Clone *A* is very high, more than six times that of Clone *D*. The grand-parental correlations are negligible but the low grandparental

TABLE 23  
*Ancestral correlations for size*

	COEFFICIENTS OF CORRELATION	COEFFICIENTS OF REGRESSION
Clone <i>D</i>		
Parent and offspring.....	0.058±0.035	0.056
Grandparent and grandchildren.....	0.018±0.036	0.020
Clone <i>A</i>		
Parent and offspring.....	0.358±0.030	0.285
Grandparent and grandchildren.....	0.030±0.036	0.048
Clone <i>A 1A</i>		
Parent and offspring.....	0.009±0.106	
Clone <i>A</i> —( <i>A 1A</i> )		
Parent and offspring.....	0.255±0.063	

correlation of Clone *A* shows that the high parental correlation is not wholly the result of the mixture of two races in Clone *A*. Computation of the parental correlation for the two subordinate clones confirms this, giving the following coefficients:

	<i>Coefficient of correlation</i>
Clone <i>AIA</i> .....	0.009±0.106
Clone <i>A</i> -( <i>AIA</i> ).....	0.255±0.063

After this division the correlation is still significant and is four times as great as that found for Clone *D*. An examination of the data for possible environmental causes of correlation

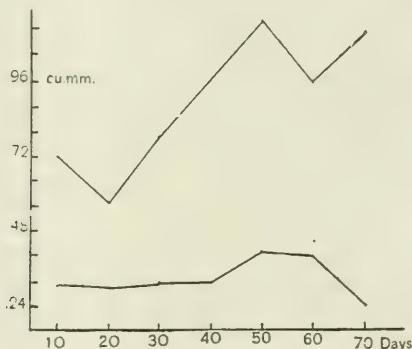


Fig. 10 Changes in the average size of the buds produced during successive ten-day intervals by Clones *A* and *D*. The ordinates represent the mean sizes in cubic millimeters of all measured buds produced during each ten-day period; the abscissae, the successive ten-day periods.

gives the result shown in figure 10. As in the case of the number of tentacles, there was a considerable change in the mean size of the buds produced at different times during the experiment.

The correlations between all buds produced in each of the five-day intervals were:

	<i>Coefficient of correlation</i>
Clone <i>D</i> .....	0.0118
Clone <i>A</i> -( <i>AIA</i> ).....	0.0952

There is here the same difference in the size of the correlations of the two clones which appeared in the parental correlation but

the correlation due to the changing clonal mean is too small to account for the parental correlation. No adequate data for a further analysis of the correlation in size is at hand, and it may be that variation in size within the clone is actually inherited but, although conclusive evidence against this is lacking, too much trust should not be placed in the parental correlations.

The following points, while based only upon general impressions gained during the experiments and subject to correction by further experimental test, indicate some of the factors which may have been instrumental in producing a high parental correlation for size. Size is a character which is modified much more readily and quickly than the number of tentacles by changes in the environment. Hydras, placed in a 0.1 per cent solution of NaCl, in a very few days become dark in color, small, and produce small, dark-colored buds. Similar changes in size follow extreme changes in temperature. Upon the restoration of optimum conditions the normal size is resumed very quickly. Starvation is effective in much the same way but to a lesser extent. When a Hydra has been injured slightly, or has passed through a slight depression it grows smaller and produces small buds but eventually both buds and parents resume the normal size of the clone. The size, indeed, seems to be a matter of the immediate state of nutrition of the polyps. Clone *A* was more readily modified by such agents than was Clone *D*. This is shown by the following data for the number of tentacles of polyps from mass cultures with and without food for three months:

<i>3-month mass cultures</i>	<i>With food</i>	<i>Without food</i>	<i>Difference</i>
Clone <i>A</i> .....	6.80±0.06	5.52±0.04	1.28±0.07
Clone <i>D</i> .....	5.52±0.05	5.34±0.03	0.18±0.06

and by the changes represented in figure 10. Corresponding to this fact, the correlations between the relatives in Clone *A* are higher than in Clone *D*, which justifies the suspicion that the correlation between parent and offspring for size within the clone is really due only to the action of the environment.

## V. DISCUSSION OF RESULTS

The experiments reported show that populations of *Hydra viridis* consist of races which have different hereditary constitutions. The diversity between two such strains persisted for so long as they were kept under observation (143 days) and so long as the different strains were kept under similar and favorable conditions they showed no tendency to approach each other in character. The distinguishing characters of the different races studied were not, however, fixed in the sense of remaining constant through fluctuations in the environment but underwent changes corresponding to changes in the environment. In general, the diverse clones responded to such changes in the same way and to the same degree, although in the face of very unfavorable conditions the larger strain was most affected and under conditions of almost complete starvation the strains became much more similar.

Little evidence has been obtained as to the cause and fundamental nature of the difference between the clones. The diverse characters noted, number of tentacles, size, color, and age at which asexual reproduction is begun, may all be modified by changing the environmental conditions and the changes thus produced in the first three characters mentioned are correlated in the same way that they are in the diverse races (large size, many tentacles, and light color occur together) so that it is possible that the diversities in these three characters are due to a difference in some single set of physiological processes. Failure to obtain sexual reproduction in *H. viridis* has made it impossible to determine the relation of the diverse races to gametic processes and to the supposed life cycle of *Hydra* but there is certainly no relation between the diversity of the clones and the phenomena of 'depression' which have been thought to mark periods in the life cycle.

The existence of diverse races of *Hydra* is in accord with the results of Jennings, Woltereck, Shull, Whitney and Agar gained from the study of clones of other invertebrates and, indeed, the volume of evidence from zoological and botanical literature

leaves no doubt that the existence of diverse races within the species is a general condition in all phyla.

The problem of inheritance of variations within the clone presents much greater difficulties and there is much conflict between the results of different investigators. The work of Whitney has shown that in *Hydatina* diverse strains may arise in a clone descended from a single fertilized egg and Calkins and Gregory report similar results for *Paramecium*. In these cases there is, however, no intimation that the inheritance of variations is a general characteristic of asexual reproduction or that the change is the result of the accumulation of slight variations. The four studies in which there is an appearance of inheritance of continuous variations within the pure line or clone are those summarized by Pearson in 1910; the studies of Johannsen on beans, Warren on *Daphnia* and *Hyalopterus*, and Hanel on *Hydra*. In all this work the evidence for inheritance can be drawn only from the ancestral correlations, while the evidence from the effects of selection seems to point the other way. The question of the relative values of the coefficient of correlation and of selection experiments hence becomes of great importance.

In Agar's recent study of inheritance in parthenogenesis, where great precautions were taken to rule out the influence of environmental agents, there is no significant correlation between parent and progeny within clones of Cladocera and sufficient evidence of environmental causes of correlation is presented to account for Warren's results with *Daphnia*. For Aphids he finds, with Warren, a slight ancestral correlation but the evidence for an environmental cause of this correlation, while perhaps not absolutely conclusive, is sufficient to make the ancestral correlation in these forms of very doubtful value as an index of inheritance. Ewing's selection experiments with *Aphis avenae* offer further evidence against the inheritance of variations in these forms.

The results recorded in the present paper are quite in accord with those of Agar on Cladocera and show that for *Hydra* also the ancestral correlation is untrustworthy as a measure of inheritance.

There remain only Johannsen's data on *Phaseolus* giving evidence of an ancestral correlation within the pure line. The evidence adduced by Pearson indicates only a slight correlation here and the recent work of Harris ('12) upon the transmission of the effects of unfavorable cultural conditions indicates that the real cause of this correlation is likewise environmental.

#### VI. SUMMARY

1. Populations of *Hydra* consist of hereditarily distinct strains which differ in initial number of tentacles, size of body, color, age at which asexual reproduction is begun, and perhaps in other characters.
2. In the absence of selection these strains remain distinct.
3. Within populations there is a correlation between the characteristics of parents and progeny and of other close relatives, which is largely due to the existence of these diverse strains.
4. Within the clone there is no significant correlation between the variations of close relatives in the initial number of tentacles.
5. Within the clone there is a slight correlation between the number of tentacles of the buds and the number of tentacles which their parents bear when each bud is produced.
6. Diversities of environment tend to produce like variations in parents and offspring and this likeness tends to disappear when the environmental cause is removed. The existence of such environmental agents is sufficient to account for the ancestral correlations found, even though there is no inheritance of variations.
7. Continued selection of variates in tentacle number results in changes in the vigor of the selected groups. This results in an apparent diversity of the differentially selected groups but the diversity persists only during selection and disappears at once when selection is discontinued. Variations in the number of tentacles of *Hydra viridis* are not inherited.
8. There is a positive correlation between the variations in the size of parent and offspring within the clone. No statistical evidence of an external cause of this correlation is presented

but from general considerations it seems probable that this, like the correlation of variations in the number of tentacles, is due wholly to the similar action of environmental agents upon parent and offspring.

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## APPENDIX

The correlation tables from which the principal constants given in the body of the paper were obtained are appended here. In all cases where the tables include an earlier and a later generation the ascendants are arranged in the rows, the descendants in the columns. In those cases where there is no qualitative difference between the members of pairs, as when siblings are correlated, the pairs are entered only once, according to the method recommended by Jennings ('11). The tables from which the correlations for size were computed are not included because of their great bulk and the lack of certain evidence upon the environmental modification of size.

TABLE 24

*Clone D: Correlation between the initial numbers of tentacles of parents and offspring*

	4	5	6	7	8	
4		12	17	1		30
5	23	126	233	7	5	394
6	76	193	542	49	23	883
7	6	37	41	2		86
8	1	1				2
	106	369	833	59	28	1395

$$r = 0.0038 \pm 0.018$$

TABLE 26

*Clone D: Correlation between the numbers of tentacles of members of the same fraternity*

	4	5	6	7	8	
4	6	133	273	24		436
5		896	3503	290	12	4701
6			4550	969	31	5550
7				77	2	79
	6	1029	8326	1360	45	10766

$$r = 0.077 \pm 0.006$$

TABLE 25

*Clone D: Correlation between the initial numbers of tentacles of grandparents and grandchildren*

	4	5	6	7	8	
4	2	9	27			38
5	16	110	242	7		375
6	84	211	505	11	5	816
7	11	13	51	1		76
8			1	1		2
	113	344	826	19	5	1307

$$r = -0.0495 \pm 0.018$$

TABLE 27

*Clone D: Correlation of the initial number of tentacles of the offspring with the number of tentacles borne by the parents when each bud was produced.*

	4	5	6	7	8	
4		9	19	2		30
5	6	95	267	21	5	394
6	8	119	623	110	23	883
7		32	45	9		86
8			1	1		2
	14	255	955	143	28	1395

$$r = 0.096 \pm 0.016$$

TABLE 28

*Clone A-(A1A): Correlation between the initial number of tentacles of parent and offspring.*

	5	6	7	8	9	
4		3				3
5	1	18	17	6		42
6	14	140	109	31	2	296
7	23	222	133	67	10	455
8	4	31	13	13	1	62
9		1				1
	42	412	275	117	13	859

$$r = 0.0011 \pm 0.023$$

TABLE 30

*Clone A: Correlation of the initial number of tentacles of the offspring with the number of tentacles borne by the parents when each bud was produced*

	5	6	7	8	9	10	
4	3	5		1			9
5	7	40	32	11	3		93
6	24	255	182	101	23	5	590
7	16	125	221	159	63	5	589
8	3	9	28	21	8	9	69
9			1	2			3
	50	432	468	293	100	10	1353

$$r = 0.240 \pm 0.009$$

TABLE 32

*Clone D'. Correlation between the initial numbers of tentacles of parents and offspring*

	4	5	6	7	
4			1	1	2
5	1	2	14	12	29
6	3	7	67	37	114
7	3		3	1	7
8	1				1
	8	8	85	51	153

$$r = -0.242 \pm 0.051$$

TABLE 29

*Clone A1A: Correlation between the initial number of tentacles of parent and offspring*

	4	5	6	7	8	
4			3	1		4
5	1	9	32	8		50
6	4	61	177	32	4	278
7		31	62	9	2	104
8			2	1		3
	5	101	276	51	6	439

$$r = -0.0342 \pm 0.032$$

TABLE 31

*Clone A: Correlation between the initial number of tentacles of grandparents and grandchildren*

	5	6	7	8	9	
4	2	1	1	2		6
5	4	24	15	15	3	61
6	39	139	139	104	12	433
7	36	53	155	219	47	510
8	8	7	18	48		81
9			2	1		3
	89	224	330	389	62	1094

$$r = 0.229 \pm 0.019$$

TABLE 33

*Clone A': Correlation between the initial numbers of tentacles of parents and offspring*

	6	7	8	9	
5	1	2			3
6	3	14	11	5	33
7	24	78	34	12	148
8		10	7	3	20
	28	104	52	20	204

$$r = 0.031 \pm 0.047$$

TABLE 34

*Clone A: Correlation between the numbers of tentacles of all buds produced in each arbitrary five-day period. Each bud is paired in the table with all the others produced in the same period*

	4	5	6	7	8	9	
4	2	82	355	270	20		729
5		574	5572	4300	484	4	10934
6			17277	35967	3859	102	57205
7				20742	4982	254	25978
8					264	30	294
9						1	1
	2	656	23204	61279	9609	391	95141

$$r = 0.0774 \pm 0.0015$$

TABLE 35

*Clone A: Correlation of the numbers of tentacles of all buds produced in each five-day period with all buds produced in the preceding five-day period. Each bud is paired individually with all buds produced in the preceding period*

	4	5	6	7	8	9	
4	9	87	374	372	42	1	885
5	66	846	4999	4552	461	5	10929
6	245	5084	32259	33039	3897	81	74605
7	153	3973	32873	38143	4650	94	79886
8	16	495	3866	4352	527	9	9265
9		4	55	93	11		163
	489	10489	74426	80551	9588	190	175733

$$r = 0.048 \pm 0.001$$

TABLE 36

*Clone D: Correlation between the numbers of tentacles of all buds produced in each arbitrary five-day period; arranged as in table 34*

	4	5	6	7	8	
4	91	1845	2936	261	12	5100
5		8993	33381	1993	72	44439
6			41613	9265	484	51362
7				797	168	965
8					6	6
	91	10838	77930	12271	742	101872

$$r = 0.1313 \pm 0.0014$$

# THE EFFECTS OF CERTAIN SALTS, AND OF ADAPTA- TION TO HIGH TEMPERATURES, ON THE HEAT RESISTANCE OF PARAMECIUM CAUDATUM

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ONE FIGURE

## EFFECTS OF SALTS ON HEAT RESISTANCE

The effects of certain salt solutions in increasing the resistance of various animals to heat are sometimes quite marked, and it seems probable that all animals of aquatic habit would be similarly affected under the proper conditions. Loeb and Wasteneys (*Jour. Exp. Zool.*, vol. 12, p. 543), found that salts exerted a great influence on the ability of Fundulus to withstand sudden changes of temperature. The maximum temperature into which these fish could, with impunity, be transferred suddenly, varied with the concentration of the sea-water or of a Ringer solution, "being about 25°C. for a concentration of M/128 or M/64; 27°C. for a concentration of M/32; 31°C. for a concentration of M/8; and almost 33°C. for a concentration of M/4." Dextrose solutions were found to lack this protective effect against a sudden rise of temperature.

In a previous paper (*Jour. Exp. Zool.*, vol. 15, p. 143) the writer mentioned a few experiments in which the heat resistance of Paramecium caudatum was increased when the animals were transferred to solutions of  $\text{NaNO}_3$ ,  $\text{NaCl}$ , and  $\text{KNO}_3$ , the whole death temperature curve being shifted two degrees higher on the scale.

The results of experiments of this kind have never been satisfactorily interpreted, and there is still lacking a complete explanation of the protective action of salts against heat, and of the acclimatizing effect of exposure to moderately high temperatures. Loeb and Wasteneys from their experiments with Fundulus in

sea-water, in Ringer solutions, and in  $\text{CaCl}_2$  solutions, conclude that the protective action of these salts is not an osmotic effect, nor a case of antagonistic salt action, but "a specific effect of the salts of the sea water." But we are left in doubt as to just what this specific effect is. Indeed the results of experiments described below seem to indicate that, in the case of Paramecium at least, the salts have no specific action, or if so, such specific action depends upon the nature of the medium in which the animal has previously lived. No attempt is made to offer any complete explanation. It is desired merely to set forth the facts as we found them as a modest contribution to the knowledge of the heat-resisting properties of living cells, with the hope that from an ever-increasing mass of data some general law may eventually be worked out.

The following experiments were carried out during the winter of 1912-13 while working at the University of Pennsylvania under the direction of Dr. M. H. Jacobs. Pure lines of *Paramecium caudatum* were used throughout, and the method of testing their resistance to heat was the same as that previously described (*Jour. Exp. Zool.*, vol. 15, p. 133-134). In testing the effects of salt solutions a small quantity of the medium containing the animals was centrifuged and two drops of the dense mass of animals were transferred with a pipette to 10 cc. of the solution in question. Five drops of this solution containing the animals were placed in each of the small glass dishes used. The dishes were covered and arranged in order on the floor of the blood-serum oven. The experiments were always conducted so that the rise from room temperature to  $45^{\circ}\text{C}.$  was accomplished in about one hour. Beginning at  $37^{\circ}\text{C}.$ , or lower if necessary, the dishes were removed consecutively, one for each rise of one degree Centigrade. After at least one-half hour, for possible recovery, the number of living and dead in each dish was counted and the percentage calculated. From the percentages the curves for the fatal temperature zones and the mean for each curve were worked out in the manner described in a previous paper. Two pure lines of *Paramecium* were studied in some detail, the one growing in a medium of alkaline reaction, and

the other in an acid medium. The results will be described separately and then compared.

*Paramecium caudatum in alkaline medium.* The medium was prepared by boiling 20 grams of hay in 500 cc. of tap-water for five minutes. Two days later, i.e., about the time of maximum acidity, just enough N/20 NaOH was added to render the fluid neutral to litmus. The culture was then seeded with a single individual isolated from a pure line previously grown in the laboratory for some weeks. The animals developed rapidly and the culture remained densely populated for about four months. The medium soon became dark brown in color and decidedly alkaline to litmus, and retained its alkaline reaction throughout its history. The effect of NaCl solutions was tested when the culture was about ten weeks old. Control experiments were carried out in every case at the same time, using five drops of the unchanged culture medium in each dish. The results obtained with M/100 NaCl are summarized in table 1.

Experiments with M/50 NaCl were carried out when the culture was about three months old; the results are summarized in table 2.

TABLE 1

*Effects of M/100 NaCl on the heat resistance of *P. caudatum* from an alkaline medium*

IN M/100 nacl: SUM OF THREE EXPERIMENTS	TEMPERATURES					
	38°	39°	40°	41°	42°	43°
Total subjected to each temperature...	333	322	279	374	365	
Number of deaths.....	0	0	19	149	365	
Percentage dead at given temperature..	0	0	7	40	100	
 CONTROLS: UNCHANGED MEDIUM, THREE EXPERIMENTS						
Totals subjected to each temperature..	480	514	463	562		
Number of deaths.....	0	6	248	562		
Percentage dead at given temperature..	0	1.1	53.5	100		
 MEANS OF THE ABOVE FATAL TEMPERATURE ZONES						
in M/100 NaCl.....				42.03°		
in controls.....				40.9°		

TABLE 2

*The effect of M/50 NaCl on the heat resistance of *P. caudatum* from an alkaline medium*

IN M/50 nacl: SUM OF FOUR EXPERIMENTS	TEMPERATURES						
	37°	38°	39°	40°	41°	42°	43°
Totals subjected to each temperature.....	95	266	370	372	380	453	430
Number of deaths.....	0	0	0	0	0	200	430
Percentage dead at given temperature.....	0	0	0	0	0	44	100
CONTROLS: UNCHANGED MEDIUM FOUR EXPERIMENTS							
Total subjected to each temperature.....	347	555	467	475	581	555	
Number of deaths.....	0	0	24	109	490	555	
Percentage dead at given temperature.....	0	0	5.1	23	84.3	100	
MEANS OF THE ABOVE FATAL TEMPERATURE ZONES	{ in M/50 NaCl.....						42.1°
	{ in controls.....						40.3°

It will be noted from the tables that M/100 NaCl has raised the mean of the fatal temperature zone of this pure line 1.13°C., and that M 50 NaCl has raised it 1.8°C., the stronger concentration producing a slightly greater effect. Concentrations stronger than M 50 had a toxic effect on this race, deaths occurring at much lower temperatures than the controls. The effect of KCl was not studied except that it was found in one experiment that M 200 KCl had a toxic effect, all the animals being dead at 38°C. In weaker concentrations it would probably have shown some protective action.

Solutions of CaCl<sub>2</sub> were tested on this same race, and weak concentrations had a marked protective action. When transferred to M 300 CaCl<sub>2</sub> nearly all the Paramecia died within two hours at room temperature, while in M 3000 CaCl<sub>2</sub> this race remained alive at room temperature for at least two weeks. Table 3 shows the effect of CaCl<sub>2</sub> alone, and table 4, the effect of a

combination of NaCl and CaCl<sub>2</sub>. When the Paramecia were transferred to a medium consisting of 10 cc. of M/100 NaCl plus one drop of M/100 CaCl<sub>2</sub> the results shown in table 4 were obtained.

TABLE 3

*The effect of M/3000 CaCl<sub>2</sub> on the heat resistance of P. caudatum from an alkaline medium*

IN M/3000 cacl <sub>2</sub> : SUM OF FOUR EXPERIMENTS	TEMPERATURES					
	39°	40°	41°	42°	43°	44°
Totals subjected to each temperature..	286	340	276	357	319	320
Number of deaths.....	0	0	0	194	310	320
Percentage dead at given temperature..	0	0	0	54.2	97.1	100
CONTROLS: UNCHANGED MEDIUM, FOUR EXPERIMENTS						
Totals subjected to each temperature..	444	409	316	554		
Number of deaths.....	0	58	314	554		
Percentage dead at given temperature..	0	11.7	99.3	100		
MEANS OF THE ABOVE FATAL TEMPERATURE ZONES	$\left\{ \begin{array}{l} \text{in M/3000 CaCl}_2 \dots \dots \dots \dots \dots \\ \text{in controls} \dots \dots \dots \dots \dots \end{array} \right.$					

TABLE 4

*The effect of NaCl plus CaCl<sub>2</sub> on the heat resistance of P. caudatum from an alkaline medium*

IN nacl + cacl <sub>2</sub> : SUM OF THREE EXPERIMENTS	TEMPERATURES					
	39°	40°	41°	42°	43°	44°
Totals subjected to each temperature..	237	228	298	391	249	235
Number of deaths.....	0	0	0	31	188	235
Percentage dead at given temperature..	0	0	0	7.9	75.5	100
CONTROLS: UNCHANGED MEDIUM, THREE EXPERIMENTS						
Totals subjected to each temperature..	312	294	316	369		
Number of deaths.....	0	58	312	369		
Percentage dead at given temperature..	0	19	98.7	100		
MEANS OF THE ABOVE FATAL TEMPERATURE ZONES	$\left\{ \begin{array}{l} \text{in NaCl + CaCl}_2 \dots \dots \dots \dots \dots \\ \text{in controls} \dots \dots \dots \dots \dots \end{array} \right.$					

The tables show that in this race the solution of M/3000 CaCl<sub>2</sub> has raised the mean of the fatal temperature zone 1.68°C. This is very nearly the same as the effect of M/50 NaCl, as shown in table 2. The most pronounced effect was produced by the addition of a little CaCl<sub>2</sub> to the solution of NaCl. In this case the mean was raised 2.26°C. The significance of these increases is all the more important when one notices that the means of the control experiments are almost exactly the same in all the tests, although they were made at very different periods in the history of the culture.

The effect of  $\text{KNO}_3$  on this race of Paramecium is summarized in table 5.

The mean death temperature of this race was 1.9°C. higher in M 50 KNO<sub>3</sub> than in the corresponding controls. In three experiments with M 100 KNO<sub>3</sub> the mean was found to be 41.1°, a rise of 0.3°C. above the corresponding controls, which gave a mean of 40.8°C.

TABLE 5

*The effect of M/50 KNO<sub>3</sub> on the heat resistance of *P. caudatum* from an alkaline medium*

		TEMPERATURES						
IN M/50 KNO <sub>3</sub> : SUM OF FIVE EXPERIMENTS		37°	38°	29°	40°	41°	42°	43°
Totals subjected to each temperature.....		295	781	634	623	735	694	691
Number of deaths.....		0	0	0	27	190	210	691
Percentage dead at given temperature.....		0	0	0	4.3	25.8	30.2	100
IN CONTROLS: SUM OF FIVE EXPERIMENTS								
Totals subjected to each temperature.....		224	380	584	677	723	725	
Number of deaths.....		0	0	221	500	723	725	
Percentage dead at given temperature.....		0	0	39.5	73.8	100	100	

Perhaps the most curious results of all were obtained when this race was subjected to the influence of M/600  $\text{Na}_2\text{CO}_3$ , and to distilled water. Table 6 shows these results and those of the corresponding controls, all of which were carried out at the same time.

The results in table 6 show that distilled water was just as effective in its protective action for this race as was M/600  $\text{Na}_2\text{CO}_3$ , or M/50  $\text{KNO}_3$ , or as M/3000  $\text{CaCl}_2$ . The mean death temperature in distilled water was  $1.78^\circ\text{C}$ . higher than the corresponding controls.

In cane sugar solutions there was no protective action manifest. In one experiment two drops of the medium densely populated with Paramecia were transferred to 5 cc. of M/8 cane sugar. When subjected to heat some deaths occurred at  $38^\circ$  and above. This solution was much less effective than distilled water; indeed, it appeared to have a weakening effect.

TABLE 6

*The effect of M/600  $\text{Na}_2\text{CO}_3$ , and of distilled water, on the heat resistance of *P. caudatum* from an alkaline medium*

	IN M/600 $\text{Na}_2\text{CO}_3$ : SUM OF TWO EXPERIMENTS	TEMPERATURES				
		39°	40°	41°	42°	43°
Totals subjected to each temperature.....	145	147	132	171	151	
Number of deaths.....	0	6	31	105	151	
Percentage dead at given temperature.....	0	4	23.3	61.4	100	
 IN DISTILLED WATER: SUM OF TWO EXPERIMENTS						
Totals subjected to each temperature.....	147	137	165	148	188	
Number of deaths.....	0	0	4	97	188	
Percentage dead at given temperature.....	0	0	2.4	65.5	100	
 CONTROLS: UNCHANGED MEDIUM, TWO EXPERIMENTS						
Totals subjected to each temperature.....	260	216	260	209		
Number of deaths.....	0	115	241	209		
Percentage dead at given temperature.....	0	53.2	92.7	100		
MEANS OF THE ABOVE FATAL TEMPERATURE ZONES		{ in M/600 $\text{Na}_2\text{CO}_3$ ..... 41.62° in distilled water..... 41.82° in controls..... 40.04°				

The above results agree with those of Loeb and Wasteneys, in one respect at least, in that they show that the protective action of salt solutions is not an osmotic effect. But when we find distilled water just as effective as three of the salt solutions used and almost as effective as M 50 NaCl, and the combination of NaCl and CaCl<sub>2</sub>, it is apparent that in the case of Paramecium at least, the results are not due to the "specific action of the salts."

Some results of tests with another race of Paramecia in an acid medium detract still more from the specific salt action as a satisfactory explanation. An account of some of these results follow.

*Paramecium caudatum in an acid medium.* The culture medium was prepared by boiling 20 grams of hay in 500 cc. of tap-water for one-half hour. The following day it was seeded with a single individual isolated from another pure culture which had been growing in the laboratory for some time. This culture medium retained its light straw color throughout. It was never as densely populated as the alkaline medium and died out sooner, i.e., in about three months. Most of the following experiments were performed when the culture was about two months old. It was still light colored and slightly acid to litmus. Tables 7 and 8 summarize the results of tests with M 4000 CaCl<sub>2</sub>, with M/100 NaCl, and with distilled water.

It is to be noted that the control experiments show that the normal heat resistance of this race was somewhat higher than that of the race from the alkaline medium, the average of all the controls of the acid medium being about one degree higher than the average of all the controls of the alkaline medium. This is just the opposite effect from that produced by acids and alkalis on the coagulation temperature of proteids. Further, it will be noted that the same salts (NaCl and CaCl<sub>2</sub>) which gave the most pronounced protective action with the Paramecia from the alkaline medium, actually decreased the resistance of those from the acid medium. Considering the action of the salts alone it might be supposed that their effects were conditioned by the reaction of the medium in which the animals had pre-

TABLE 7

*The effect of M/4000 CaCl<sub>2</sub> on the heat resistance of *P. caudatum* from an acid medium*

IN M/4000 cacl <sub>2</sub> : SUM OF TWO EXPERIMENTS	TEMPERATURES				
	39°	40°	41°	42°	43°
Totals subjected to each temperature.....	243	206	202	170	
Number of deaths.....	0	200	202	170	
Percentage dead at given temperature.....	0	97	100	100	
 CONTROLS: SUM OF TWO EXPERIMENTS					
Totals subjected to each temperature.....	238	330	143	300	295
Number of deaths.....	5	23	224	210	295
Percentage dead at given temperature.....	2.1	7	15.4	70	100
 MEANS OF THE ABOVE FATAL TEMPERATURE ZONES					
{ in M/4000 CaCl <sub>2</sub> .....				39.53°	
{ in controls.....				41.55°	

TABLE 8

*The effect of M/100 NaCl, and of distilled water, on the heat resistance of *P. caudatum* from an acid medium*

IN M/100 nacl: SUM OF THREE EXPERIMENTS	TEMPERATURES			
	39°	40°	41°	42°
Totals subjected to each temperature.....	368	308	299	327
Number of deaths.....	0	49	242	327
Percentage dead at given temperature.....	0	15.8	81	100
 IN DISTILLED WATER: SUM OF THREE EXPERIMENTS				
Totals subjected to each temperature.....	395	390	332	429
Number of deaths.....	0	72	326	429
Percentage dead at given temperature.....	0	18.5	95.1	100
 IN CONTROLS: UNCHANGED MEDIUM THREE EXPERIMENTS				
Totals subjected to each temperature.....	662	685	622	770
Number of deaths.....	0	15	190	770
Percentage dead at given temperature.....	0	2.2	30.5	100
 MEANS OF THE ABOVE FATAL TEMPERATURE ZONES				
{ in M/100 NaCl....				40.5°
{ in distilled water..				40.3°
{ in controls.....				41.1°

viously existed. But distilled water was found to have the same effect, and some influence other than the salts seems to be the important factor.

Taken as a whole, the above experiments seem to point to the conclusion that certain properties of the medium are important factors in the heat resistance of *P. caudatum*, and that such properties will predetermine whether a given salt solution will have a favorable or an unfavorable effect. Whether this is true for other Protozoa remains to be determined, and a satisfactory explanation is yet to be proposed.

#### EFFECTS OF ACCLIMATIZATION TO HIGH TEMPERATURES ON HEAT RESISTANCE

A few experiments were carried out during the same season with a view to determining to what extent continued exposure to moderately high temperatures would influence the death temperature. Several attempts along this line failed, some on account of too sudden changes and some for other reasons. Some success was had with two cultures which were studied at frequent intervals during an exposure of over two months to temperatures ranging from 28° to 36°C. Pure line cultures of Paramecia were used in these experiments.

On January 30, 1913, a culture medium was prepared by taking 35 grams of hay in two liters of distilled water. This was heated for a period of ten minutes at a temperature of about 60°C. This was then divided equally between two culture jars so that each contained one liter of the fluid and about 17½ grams of hay. After cooling, each of these cultures was seeded with a single individual taken from a pure line which had been growing in the laboratory for 86 days before this date. The two resulting cultures, which will be referred to as '30-a' and 30-b,' may therefore be regarded as of the same pure line and growing in the same medium. On February 11 (12 days after the culture was started) 30-a was transferred from room temperature to a water bath at 28°C. The temperature of the water bath was regulated automatically and never varied more than 0.5° either way. The culture jar was so placed that the level of the cul-

ture fluid inside was about one inch below the level of the water of the bath outside the jar. The mercury bulb of the thermometer showing the temperature of the water bath was also placed about one inch below the surface of the water, so that it gave very closely the actual temperature to which the Paramecia were exposed. The culture jar itself was of course kept covered with a glass plate at all times. The constant temperature of  $28^{\circ}$  was maintained for a period of 27 days. On March 10 the temperature was raised to  $30^{\circ}\text{C}$ . and on the 12th to  $32^{\circ}\text{C}$ . After 10 days at  $32^{\circ}$  the temperature was again raised to  $34^{\circ}$ . After 16 days at  $34^{\circ}$  the temperature was raised on April 7 to  $35^{\circ}$ , and on April 15 to  $36^{\circ}$ , at which point it was maintained to the end of the experiment. On April 26 the culture was in very poor condition and very few animals were present. A little dry fresh hay was added to the medium but it did not have any favorable effect and the strain had completely died out by April 29.

Strain 30-b was used as a check on 30-a, and was kept on a table in a cool room, the temperature of which varied from  $12^{\circ}$  to  $22^{\circ}\text{C}$ . On April 29, when 30-a had completely died out, 30-b was still in good condition, although not as densely populated as in earlier periods of its growth.

During the course of the experiment the heat resistance of both strains was studied at frequent intervals. The mean of the fatal temperature zone for each experiment was worked out in the usual way and these means are plotted in figure 1.

Another pure culture, 11-2, was started from an individual isolated on December 11, 1912. This culture was kept at room temperature until February 14, at which time it was transferred to the water bath at  $28^{\circ}\text{C}$ . From that time on until the culture died out (April 25) it was subjected to the same temperature conditions as was 30-a. The mean death temperatures of a series of tests with this strain are also plotted in figure 1. Examination of the figure shows that the resistance of the control was by no means constant. There was considerable variation, the means varying from  $40.5$  to  $42.3^{\circ}\text{C}$ . The resistance was more irregular and in general slightly higher during the later course of its history than in the earlier experiments.

Mean death temperatures of three races of *P. caudatum* in degrees Centigrade

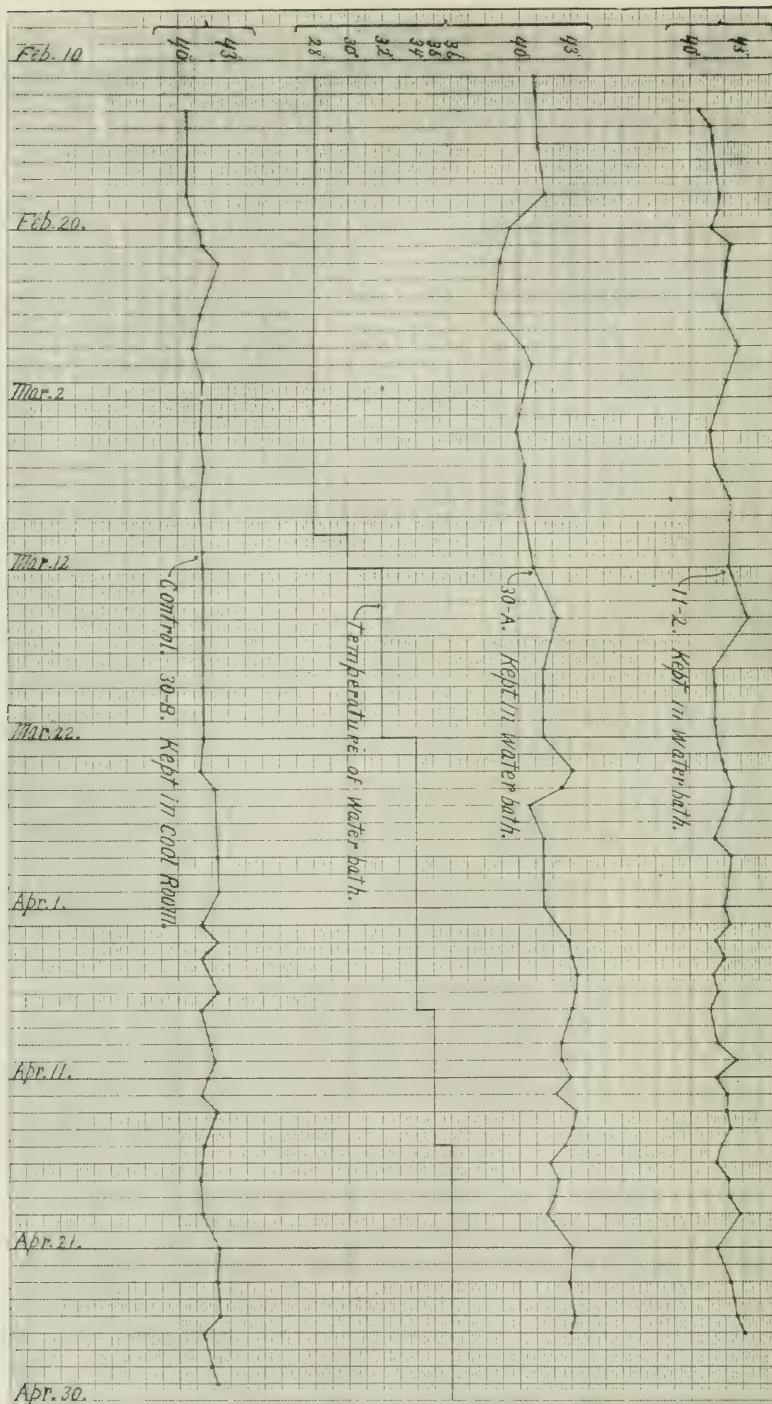


Fig. 1 Showing the mean death temperatures of two races of *Paramecium* growing at moderately high temperatures, and those of a control experiment kept in a cool room.

In the case of 30-a, which was growing in practically the same medium as the control, we see a much greater fluctuation. The mean death temperatures for this race vary from 38.5 to 43.4°C. After one week at 28° the resistance decreases and is lower than at the beginning but after February 27 the resistance increases again and the general trend of the line is gradually upward. After the temperature was raised to 34°C. the resistance of this strain remained rather high, being in general from 1 to 2.5° above its initial resistance. However, the highest mean was only 1° above the highest mean for the control culture.

Several experiments with strain 11-2 before it was put in the water bath gave an average mean fatal temperature of 40.5°C. As soon as this culture was subjected to the higher temperatures of the water bath we find that the resistance increases and remains at least 1° higher than the initial resistance and at times is more than 2° higher.

The mean death temperatures of both 11-2 and 30-a at times approached 43° and sometimes exceeded it, while, as pointed out above, the death temperature of the control, 30-b, never exceeded 42.3°. However, this increased resistance of those strains growing at the higher temperatures was not maintained for any considerable period, and it is hard to see that any decided effect was produced.

#### SUMMARY

1. The effects of certain salt solutions on the heat resistance of *Paramecium caudatum* were tested. Two pure lines of *Paramecia* were used; one growing in a medium of decided alkaline reaction, and the other in a slightly acid medium. Experiments with the race from the alkaline medium showed that M 100 and M 50 NaCl, M 3000 CaCl<sub>2</sub>, and M/50 KNO<sub>3</sub> exerted a marked protective action. The greatest increase was noted in a solution of M 100 NaCl to which a little CaCl<sub>2</sub> had been added. Distilled water also was found to increase the heat-resisting powers of this race.

2. The Paramecia from the acid medium were adversely affected by M 4000 CaCl<sub>2</sub>, by M 100 NaCl, and also by distilled water.

3. The conclusion is drawn that certain properties of the medium in which the animals had been growing were the important factors in determining the ability of Paramecium to withstand heat. No explanation is offered as to what these factors are, nor how they act.

4. The effects of continued exposure to moderately high temperatures on the death temperature of Paramecium were studied. Two cultures of Paramecia were kept in a water bath, the temperature of which ranged from 28° to 36°C. The mean death temperature of these two strains fluctuated considerably, and at times was about 1° above the highest mean death temperature of a control culture kept in a cool room. But this increase above the control was not constant, and on the whole no very decided effect was produced.

## DIDINIUM NASUTUM

### I. THE LIFE HISTORY

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TWELVE FIGURES—ONE PLATE

The work of Woodruff and Erdmann ('14) on *Paramecium aurelia* showing the occurrence of periodic reorganization of the cell, which, like conjugation, has the effect of renewing vitality, raises the question as to the length of life of a ciliated protozoon and its progeny in which both asexual reorganization and conjugation are prevented. Fermor ('13) has shown that a similar reorganization occurs in *Stylonichia* during the process of encystment. Prandtl ('06) made the statement, unsupported by evidence, however, that in *Didinium nasutum* nuclear reduction occurs during encystment as well as during conjugation. These observations indicate that encystment in ciliates, when not for purposes of protection against adverse environmental conditions or for division (as in *Tillina*), is a process during which nuclear reorganization, or parthenogenesis, takes place. An encysting organism in which asexual endomixis takes place has advantages over *Paramecium* in the present problem because of the definite external advertisement of the internal processes taking place.

*Didinium nasutum* was chosen for the experiments because of its large size, its easily controlled feeding habits and because of its readiness to encyst. The feeding habits have been worked out by Mast ('09) and the process of conjugation by Prandtl ('06).

### MATERIAL AND METHOD

Two individuals—X and Y—of *Didinium nasutum* were isolated from fresh material brought into the laboratory from Van Cortlandt lake on October 28th, 1914. They were placed in ground-glass flat-bottomed culture dishes each containing 0.25

cc. of clear pond water. Twelve individuals of *Paramecium caudatum* were picked out and placed with each of them, this number being purely arbitrary. After a few days' trial it was found that a smaller number of *Paramecium* gave better results and a standard daily diet of 9 *Paramecium caudatum* was established and maintained throughout the experiments, which are still under way. Five lines of X and five of Y were established on the second day and one individual from each of the ten lines was picked out and placed with 9 *Paramecium caudatum* in 0.25 cc. fresh spring water daily. The usual history of *Didinium* in such an environment at the end of twenty-four hours is 8 *Didinium* and no *Paramecium*. At times we find only 2 or 4 *Didinium* and no *Paramecium*, showing that the appetite was good but the dividing power reduced. Again we find occasionally 2 or 4 *Didinium* and from 2 to 5 *Paramecium*, or sometimes, only 1 *Didinium* and from 8 to 10 *Paramecium*, indicating what I shall speak of as loss of appetite. In still other cases the single individual does not divide at all but, notwithstanding daily changes of water and food, dies, usually by the fourth day. Finally encysted individuals which have not divided are occasionally found at the end of twenty-four hours, together with from 9 to 13 *Paramecium*. The rate of division of *Paramecium* is of course very low owing to the scarcity of bacterial food.

#### GENERAL DESCRIPTIONS

##### *Feeding habits of Didinium nasutum*

Next to the capture of *Halteria grandinella* by *Actinobolus radians* I know of nothing more spectacular or amazing in the whole realm of microscopy than the seizure and ingestion of *Paramecium* by *Didinium*. Described by Balbiani ('73), by Thon ('05), by Jennings ('06) and by Mast ('09) there is little in the process for me to dwell on. The actively rotating carnivore swims vigorously through the water, occasionally limiting its activity to side or bottom of the culture dish, making vicious jabs downwards or sideways until it hits something soft enough for its proboscis to penetrate. As earlier observers have pointed

out, there is no evidence whatsoever of choice of food nor any evidence of chemiotactic guidance of captor to prey. The entire process is apparently fortuitous, some one of hundreds of jabs is successful and a Paramecium, once hit, rarely gets away (fig. 1). The victim is partially or wholly paralyzed and is speedily swallowed, the walls of the Didinium being stretched around the prey like a rubber bag. If the Paramecium is seized at or near one end, this end goes in first (fig. 2) until it reaches the extremity of the captor (fig. 3). It is then doubled on itself until it lies like a U completely ingested (fig. 4). The Paramecium protoplasm becomes highly vacuolated, broken up into small pieces and is quickly digested (fig. 6). If the Paramecium is seized in the middle, this part goes in first and the two ends last, so that the victim is swallowed in the U form. Not only can a small Didinium thus capture and engulf a Paramecium six times its size, as shown by Mast, but it will swallow a dividing Paramecium, and I have frequently watched one attack and swallow a pair of conjugating Paramecium. My imagination has pictured the surprise which such a Didinium might feel when, having completed its usual task, it found itself compelled to swallow another equally large meal. In such cases one of the free ends of the two victims is usually seized; this individual is ingested and the process is continued until the second individual is completely engulfed. It means a little more tension on the part of the elastic walls of the captor but, usually, he is equal to it. Such stuffed individuals are subject, however, to diffluence, especially if transferred shortly after feeding to fresh water, and I have watched more than one individual explode, victims of their gluttony.

#### *Structure of the proboscis and seizing organ*

The proboscis of Didinium is a conical projection in the center of the anterior end. It is supported by a dense layer of trichites which are anchored deep in the protoplasm. These are evidently strengthening organs and probably play a part in preventing rupture when a large food body is swallowed, in much the same

way that spiles in a ferry slip take up the strain. In the center of the conical proboscis is a column of protoplasm somewhat denser than the rest of the endoplasm. This structure, called by Thon the 'mittlerer Strang' and by Mast the 'seizing organ,' is the apparatus which fastens the prey and precedes it into the body of the captor. The open passage which it leaves by its migration through the protoplasm becomes the cytopharynx, the prey being drawn in largely, if not entirely, by its pull (figs. 1-6).

This seizing organ is such a remarkable structure that it well repays careful study. There is no doubt that it contains some toxic substance which partially or wholly paralyses *Paramecium*, but no one, as yet, has shown where this substance lies. Balbiani ('73) held that trichocysts are discharged by *Didinium* and that these penetrate and paralyse the victim. Thon, however, supported by Mast, denies the discharge of trichocysts and holds that the entire process of capture and retention is a function of the seizing organ. Thon figures the seizing organ as striated, and in this he is undoubtedly correct, but he evidently failed to note a zone of thickened granular striae near the apex of the seizing organ and clearly apparent when the organ is extended (fig. 8). This zone is made up of the same sort of thing, apparently, as the granular zone at the apex of a tentacle of *Actinobolus*. These granules were first described for *Actinobolus* by von Erlanger ('89) as trichocysts on observations confirmed by Moody ('12) who speaks of them as 'trichocyst material.' They evidently are the poison granules which cause the instantaneous paralysis of *Halteria*. In the absence of other physical evidence of poison in the seizing organ of *Didinium* we are justified in regarding these granules, liberated on penetration of the cortex of *Paramecium* or other victim, as the cause of paralysis. The entire seizing organ may be regarded as a bundle of structures homologous with the distributed tentacles of *Actinobolus*. Like the seizing organ, each tentacle of *Actinobolus* is retracted into the endoplasm, dragging the attached victim to the surface of the body, where it is manipulated by the cilia until swallowed through the mouth.

*Structures of the endoplasm*

Thon has given an excellent account of the finer structures of the endoplasm of Didinium. One or two points should be mentioned here as they have to do with structures involved in different stages of the life history. The most important of these are the nuclei and their derivatives.

The macronucleus is correctly described by Thon. In the resting stages it is characterized by deeply-staining spherical granules of chromatin embedded in a more feebly-staining matrix. These bodies in the nucleus behave during division like the chromatin bodies of *Dileptus gigas*, where they are distributed throughout the cell. At periods of division of Didinium they form first a more or less complicated reticulum by elongating and fusing at one or more points. The strings of chromatin are ultimately divided, again as in *Dileptus*. At periods of encystment the distinct granules of the nucleus become much larger and are discharged from the nuclear mass until the cytoplasm becomes filled with densely-staining chromatin bodies. After recovery from encystment the distributed chromatin masses are broken up into smaller metaplastic granules, which give a uniformly dense stain to the entire endoplasm. Finally, at conjugation, these metaplastic bodies disappear from the endoplasm and are concentrated in a deeply-staining cortical armature in the ectoplasm.

The micronuclei were entirely overlooked by Thon. They are extremely small and difficult to distinguish from the numerous spherical bodies distributed throughout the endoplasm. At periods of conjugation, however, they are plainly evident and their history may be followed with comparative ease. This was first done by Prandtl ('07), who also for the first time described the micronuclei in vegetative stages. The number, according to Prandtl, is variable, two or three being usually present and these are closely anchored to the macronucleus (fig. 7). In division one pole of the spindle is usually embedded in the substance of the macronucleus. I have confirmed these observations of Prandtl, finding as many as four micronuclei during the

resting stages of non-conjugating forms, four in organisms preparing to encyst, and as many as sixteen in the conjugating animals. Owing to the difficulty in finding them even in the most carefully stained sections, no positive statement can be made as to the 'normal' number, but it appears to be four. They are very minute ( $4-6 \mu$ ) with relatively little chromatin, usually concentrated in a few central granules, and with definite nuclear membranes. The division spindles are narrow and sharply pointed with the chromatin in minute chromosomes (fig. 7).

In addition to the nuclei there are numerous curious and enigmatical structures in the endoplasm which I am unable to interpret. These are often in the form of spindles with curious rod-like bodies which suggest chromosomes (fig. 7). These are found during all stages of vegetative life. There is no evidence of their division and no reason to believe that they are nuclei, and the only suggestion I have to offer as to their function, is their possible connection with the formation of new seizing organs to replace those used up in food capture.

#### THE LIFE CYCLE

In other places I shall describe the details of encystment and of conjugation, and will limit the present paper to the history of the race from October 28th to the present time (April 20). Thus far the organisms have gone through two completed cycles, each ending in encystment of all the living material and during which nuclear reorganization occurred.

##### *The initial cycle (October 31 to December 28): Individual X*

Five lines derived from individual X were each changed to fresh water and fed 9 *Paramecium caudatum* daily. The daily rates of division were averaged for 5-day periods and the results plotted to give the accompanying chart A. The daily records include the number of divisions which each individual had undergone during the preceding twenty-four hours, the number of living *Paramecium* in each culture dish, the number that

had encysted, and the number that had died. In case of death or encystment of an individual in any line, its place was filled from among the descendants of some other line in the same

X Series: 1st Cycle

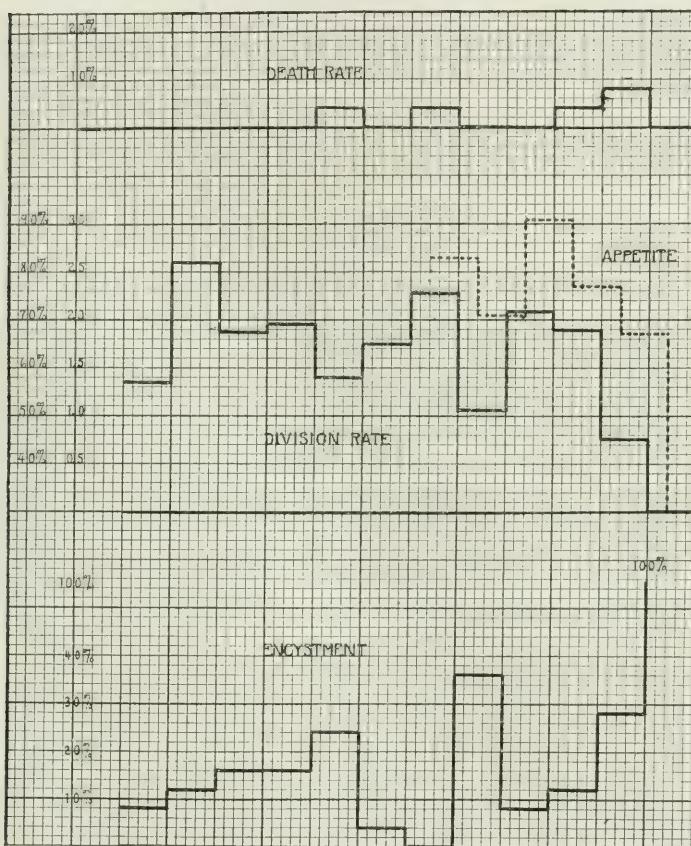


Chart A

series. Superfluous individuals each day were supplied with food and kept as 'stock' in the moist chambers.

The division rate averaged 1.95 divisions per day for the first 20 days of this cycle, 1.64 per day for the second 20 days, and

1.18 for the last 20 days, falling finally to zero with encystment of all the living material in approximately the 131st generation (chart A).

The percentage of encystment, computed from the total number of individuals to encyst in the 5-day periods and the total number of individuals under observation, is shown at the bottom of chart A. There is but little ground for comment here, the fluctuations coinciding more or less with those of the division rate. The percentage was low at the outset but increased later until it finally rose to 100 per cent.

The death rate, computed in the same way as the encystment rate, was comparatively low throughout the cycle, never rising above 8 per cent (chart A, top).

The records of the numbers of *Paramecium* eaten, determined by the numbers found alive at the end of twenty-four hours, were not begun until one month after the cultures were started. These records furnish the basis for a study of the variations in what may be termed the 'appetite' of *Didinium*, shown in the dotted line of chart A. The data for this curve were obtained as follows: In 5-day periods the five lines of culture material are provided with 45 *Paramecium* daily, or 225 during the period. Add to these 25 per cent for approximate increase by division before being eaten, giving 280 *Paramecium* for the 5-day period for all five lines. The daily records give the numbers of *Paramecium* alive at the end of twenty-four hours. These are averaged for 5-day periods and the average, divided by 280, gives the percentage of uneaten *Paramecium*, which subtracted from 100 per cent gives the approximate percentage of *Paramecium* eaten. Rough as this method is in illustrating the variations in appetite of *Didinium* the curve nevertheless follows that of the division rate with remarkable fidelity. It might be argued that the division rate should follow the eating rate and that the 5-day periods in the 'appetite' curve should be twenty-four hours in advance of the periods indicating the division rate. But it is equally true that the feeding rate depends on the vitality of *Didinium*, and as the records for feeding brought the initial dates of the periods forty-eight hours later than those for the

division rate, I have worked them out on this basis. The fluctuations of the appetite curve follow closely those of the division rate and both are correlated with the fluctuations in the curve of encystment. When the latter reaches 100 per cent both division-rate and appetite-rate fall to zero. The over-lapping at this period (Dec. 25 to Dec. 30) is due to the fact that Paramecium may be eaten prior to encystment but without division of Didinium.

*The second cycle (December 28 to March 5): Individual X*

All the living material of Didinium in culture and stock dishes became encysted during the period beginning December 25. The race was recovered from encystment December 28, by pouring off the old water and adding fresh water and Paramecium to a Syracuse dish in which stock material of the X series had encysted the week before. The division rate immediately indicated a renewal of vitality, giving an average for the first 20 days of 2.01 divisions per day. It then fell to 1.76 for the second 20 days and to 1.25 for the third 20 days. In the last 5-day period (February 28 to March 4) it averaged only 0.52 divisions per day, after which all culture material and stock material encysted (chart B). The race passed through 148 generations during this cycle, or 279 generations since the culture experiments started.

The rate of encystment began at 16 per cent for the first 5-day period but quickly fell and remained low during the first 40 days, after which it maintained an average of about 17 per cent until all individuals became encysted (chart B, bottom).

An interesting feature of this second cycle was the increase in the average death rate over the first cycle. The average death rate for the entire first cycle was 1.8 per cent and for the entire second cycle it rose to 7 per cent. When we consider the relative infrequency of death of the individuals in culture this phenomenon becomes significant (chart B, top).

As in the first cycle, the curve for appetite closely follows the curve of reproduction (chart B, dotted line).

X Series: 2nd Cycle

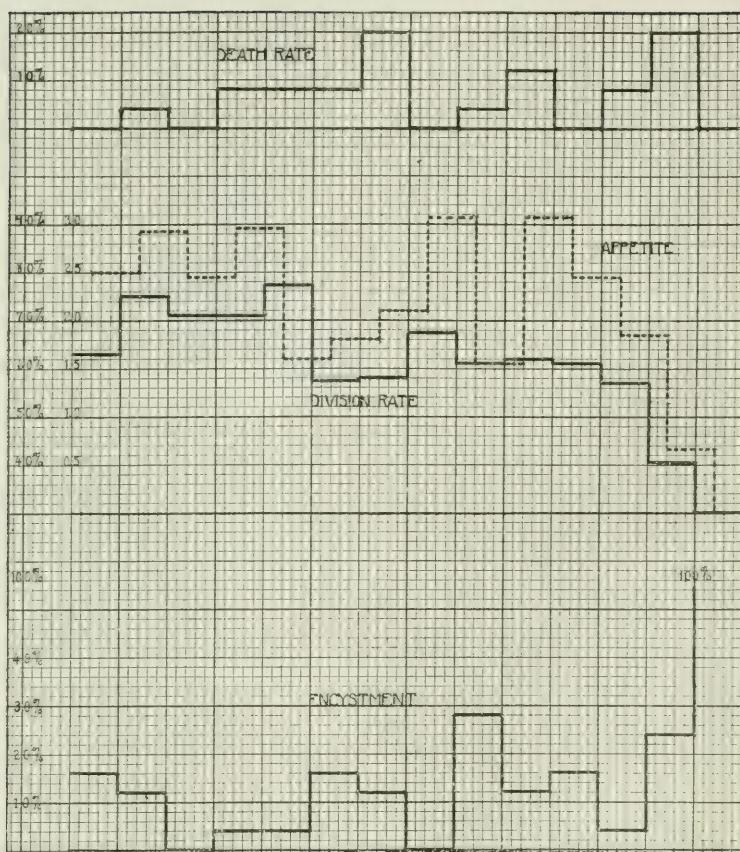


Chart B

*The third cycle (March 9 to date, April 26): Individual X*

After encystment of all individuals in culture and stock dishes the race was recovered on March 8th as before, by adding fresh water and food to a stock dish set aside on the 27th of February. By the 3rd of March all of these had encysted. Five days later 100 cysts were picked out and placed in fresh water with *Paramecium caudatum*. On the following day (March 9) there were

about 40 active Didinium in the culture dish. Five of these were isolated and furnished material for the third cycle. Twelve were killed and the remainder were fed and left as stock. All of the remaining cysts were killed for cytological study.

The average division rate during the first twenty-four hours after recovery was 3.4, and for the first 25 days it remained high (2.19 per day). The encystment rate for the entire period was 8 per cent, while the average death rate for the period of 25 days rose to 14.4 per cent. This increase in the death rate as the culture series grows older, and already indicated in the second cycle, is interesting and significant.

#### *Cultural history of the Y series: Initial cycle*

The Y series, started with a second individual at the same time as the X series and treated in the same way, confirms the results obtained with the X series. The first cycle (Nov. 1 to Dec. 26) had the same general history as in the X series but with a slightly more regular descending curve of the division rate (chart C). The average for the first 20 days was 1.65 divisions per day; for the second 20 days, 1.19 divisions per day, and for the third 20 days it fell to 0.65 divisions per day.

The curve for encystment is much more regular than that for the X series and, with one exception in the 7th 5-day period, shows a fairly steady increase (chart C, bottom). The death rate was very low (chart C, top) and the appetite curve is similar to that for the X series (chart C, dotted line).

The cycle came to an end with encystment of all culture individuals a few days in advance of the X series, and in the 128th generation, on December 26.

#### *The second cycle (December 28 to February 6)*

The race was recovered from encystment December 28 from stock material which had encysted on the 22nd, and a second cycle was started with an initial division rate of 2.08 divisions per day (chart D). The vitality was not as great as before, the average division rate for the first 20 days being only 1.65 per

Y Series: 1st Cycle

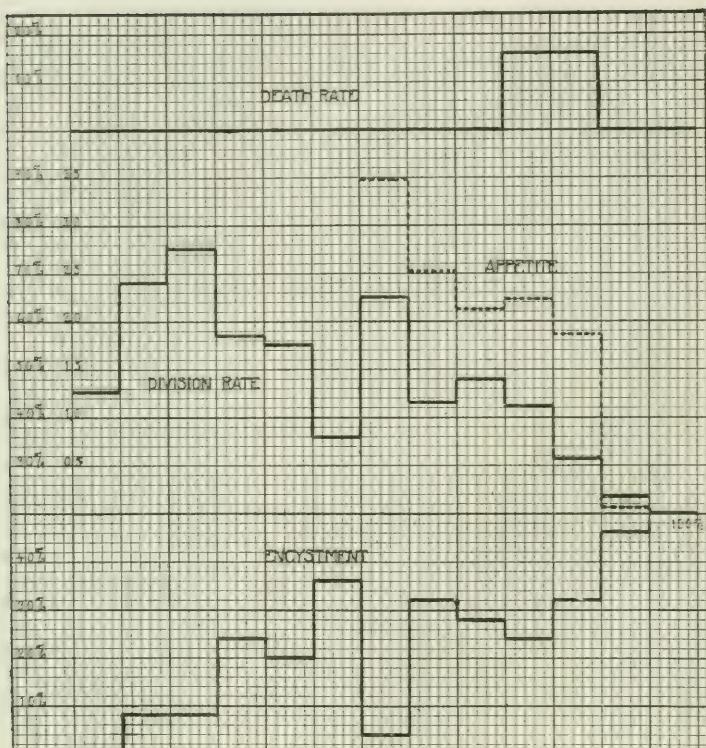


Chart C

day, and for the second 20 days only 1.05 per day, and the cycle came to an end with encystment of all living material at the end of 40 days. This cycle included only 84 generations, giving a total of 212 generations for the series in culture.

The rate of encystment was fairly high throughout this cycle and went up rapidly during the last four 5-day periods (chart D, bottom), while the death rate showed a slight increase over that of the first cycle.

Notwithstanding the relatively low division rate, the appetite was remarkably good, nearly 88 per cent of the Paramecium being eaten daily for the first 25 days (chart D, dotted line).

All efforts to recover the series from encystment failed; not one individual could be persuaded to come out of its cyst, and the race thus came to an end.

Y Series: 2nd Cycle

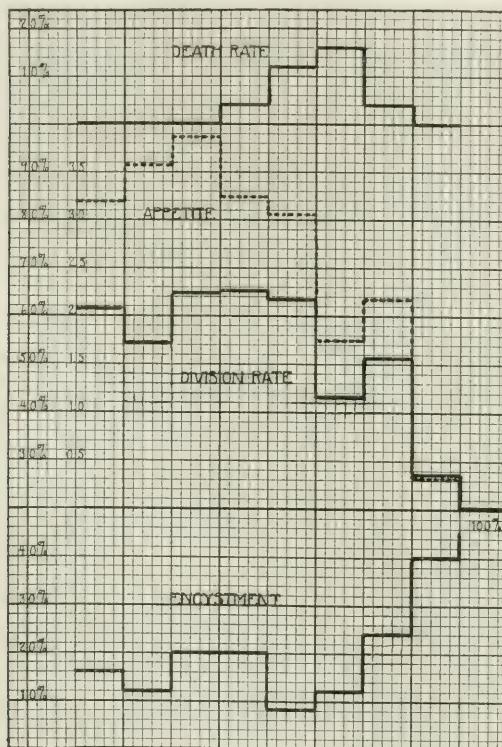


Chart D

## GENERAL

Encystment in ciliates has a three-fold purpose. First, for protection against adverse conditions of the environment, which, as Fermor has well observed, are usually so delicately adjusted to the equilibrium of an organism that they baffle detection. Such encystment is characterized by no internal reorganization and the organism may be recovered in twenty-four hours or less

by substituting fresh water for the medium in which it had encysted. Second, for reproduction, a phenomenon observed in *Tillina*, *Colpoda* and a number of other ciliates, but by no means universal in the group. Third, for reorganization, which has to do with internal processes of the cell. In *Didinium* there is no encystment for purposes of reproduction, but it is frequent for purposes of protection and periodic for purposes of reorganization. In the latter case the approach of encystment can be predicted very often from the reduced activity in feeding and in dividing, from two to four days in advance. This is shown not only by the averages for the entire race but also by individuals and their progeny watched from day to day. When in this condition, fresh water and food have no effect, nor will fresh water added daily bring such individuals out of their cysts until a period of at least five days has elapsed.

A very unexpected result was obtained in these experiments in connection with the phenomena of conjugation. During the first cycle no conjugating pairs were observed in any of the stock dishes although such material is prepared daily and always watched for at least five days. During the first week of the second cycle, epidemics of conjugation appeared in the stock dishes. This period of conjugation lasted about ten days, after which not a pair was seen. Conjugation epidemics appeared again in the third cycle and at a corresponding time. The first pairs were seen in the stock dishes on the third day after recovery from encystment (March 12) and pairings occurred in great numbers until March 20th, after which not one pair could be obtained from the material. During the height of the epidemic in the stock material two cases of conjugation occurred in the isolation cultures. One of these pairs (March 16) was the union of two individuals out of eight derived from one individual isolated the day before. The second case occurred on March 17 between two individuals among sixteen derived from an individual isolated the day before.

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## PLATE 1

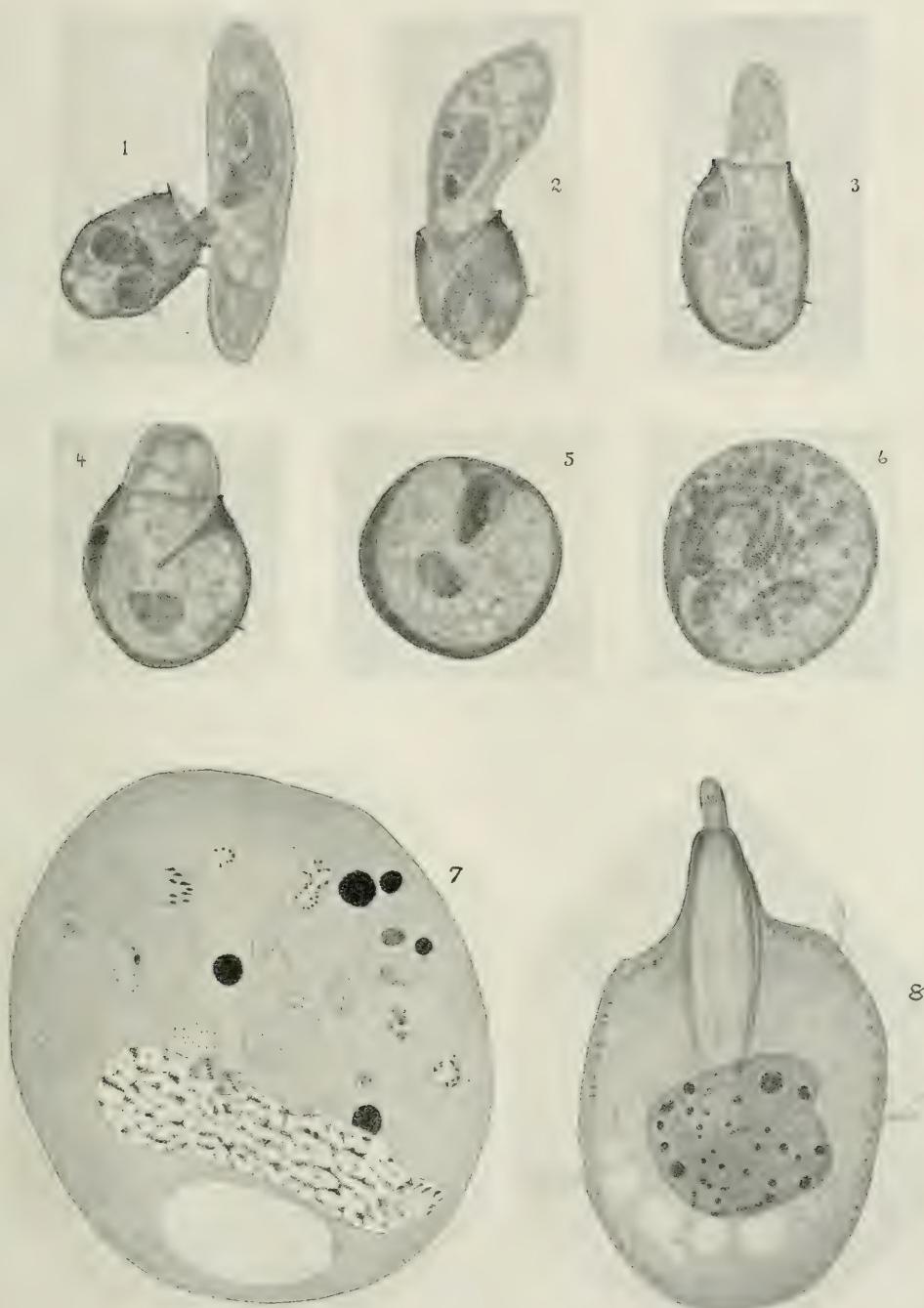
### EXPLANATION OF FIGURES<sup>1</sup>

1 to 6 Stages in the process of swallowing *Paramecium caudatum*. Camera drawings from preparations mounted *in toto*.

7 Section of dividing form of *Didinium*. The macronucleus section shows the characteristic reticulum of the early stage of division. Two micronuclei in full mitosis lie in the margin of the macronucleus; several large endoplasmic bodies are shown in section, and two enigmatical bodies, which may be seizing organs in the process of development.

8. Section of *Didinium* preparatory to encystment. The trichites of the proboscis and the basal fibrils of the membranulae are clearly shown at this stage; the peripheral protoplasm is denser than at other periods; the macronucleus shows the enlargement of the contained chromatin bodies. The micronuclei at this stage leave the hollows in the margin of the macronucleus, swell, and prepare to divide; one of the four is shown at the left of the macronucleus. The seizing organ is extruded and shows the zone of dark granules near the tip. These probably represent the poison of the 'trichocyst material' instrumental in paralyzing the prey.

<sup>1</sup> Drawn by Mabel L. Hedge from permanent preparations.





# THE REACTIONS AND RESISTANCE OF FISHES IN THEIR NATURAL ENVIRONMENT TO SALTS

MORRIS M. WELLS

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### I. INTRODUCTION

In a previous paper (Wells '15 a) the reactions and resistance of fresh water fishes to alkalinity, acidity and neutrality were discussed upon the basis of experimental evidence which seemed to indicate that the chemical reaction of the water (i.e., acid, neutral or alkaline) in which the fishes live, is a matter of considerable importance to fresh water fishes and probably to marine fishes also (Shelford and Powers '15). In the present paper a large number of experiments bearing upon the reactions and resistance of fresh water fishes to salts is presented. Practically no previous work has been published upon the *reactions* of fishes to salts and the main part of the data presented here has to do with this phase of the subject. Some interesting relations between acidity and resistance to salts are also presented. This latter phase of the subject has been worked out in a preliminary way only; the more definite relations are left for further investigation.

The present investigation was begun at the suggestion of Prof. V. E. Shelford and was carried on at the University of Chicago during the years 1912 and 1913. In the fall of 1914 operations were transferred to the University of Illinois as the author accompanied Dr. Shelford in his transfer to that place.

### II. THE WATER

The differences in the water of the two institutions have been discussed in the first paper of the series (Wells, l. c.). The chief differences are the following: The water at Chicago comes from Lake Michigan; as it flows from the tap in the laboratory, it is slightly acid with carbon dioxide (2-3 cc. per liter), is supersaturated with O<sub>2</sub> (8-10 cc. per liter), contains 32 cc. per liter of half-bound CO<sub>2</sub> (bicarbonates) and a proportionate amount of other salts. The water at the University of Illinois comes from deep wells. As it flows from the tap it is strongly acid (18 cc. CO<sub>2</sub> per liter), contains practically no O<sub>2</sub> (0.12 cc. per liter) and the half-bound CO<sub>2</sub> equals 101 cc. per liter; other salts are in proportion. Aeration brings the two waters to more nearly

the same condition and fishes can live in either after the proper amount of aeration. Too much aeration causes the Illinois water to become alkaline to phenolphthalein and fresh water fishes cannot live in such water.

### III. METHODS AND APPARATUS

The reaction experiments have been performed in the gradient tank used in the acid gradient experiments (Wells '15 a, fig. 1, p. 223).

The salts used have been, the chlorides, nitrates and sulphates, of ammonium, potassium, sodium, calcium and magnesium. In presenting the results of the reaction experiments the salts will be grouped with reference to the anion, as the similarities in behavior, in the different salt gradients, make this a rather natural division. They will also be taken up in the order of increasing toxicity of this ion as worked out by Lillie ('10) and others. Thus the order of consideration will be, chlorides, nitrates, sulphates. In considering the resistance experiments, on the other hand, the salts will be grouped according to the cation. In the gradient experiments the concentration of salt introduced at the salt end has been in nearly all cases 0.01N. In a few experiments the concentration was made 0.02N or even higher in an attempt to drive the fishes out of the salt end, to which they were giving a positive reaction. These experiments will be cited as they come up.

The gradient in the salt experiments was obtained as follows: Tap water was set to flowing into one end of the tank at the rate of 500 cc. per minute, and into the other end at the rate of 400 cc. per minute. A 0.05 N solution of the salt was made up with tap water and run into the flow at the 400 cc. end at the rate of 100 cc. per minute. This made the volume of the flow at the two ends equal. The salt solution was mixed with the tap water, in a mixing bottle, outside the experimental tank. From the mixing bottle a single outlet led to the experimental tank. At first the gradients were tested before and after each experiment. Later, after a very careful study of the gradient

had been made at Chicago, by determining the conductivity of the water at various points in the tank, tests were no longer made. Thus the actual concentrations existing throughout the tank have not been determined in each experiment but the study that was made indicated very clearly that under the given conditions this concentration is almost constant for a given salt. Thus there always exists a gradient of the dissolved salt, between the two ends of the tank. The presence of this gradient is shown by the reactions of the fishes as well as by the conductivities and titrations. That the gradient is not perfect is to be expected; its peculiarities were brought out in the study which

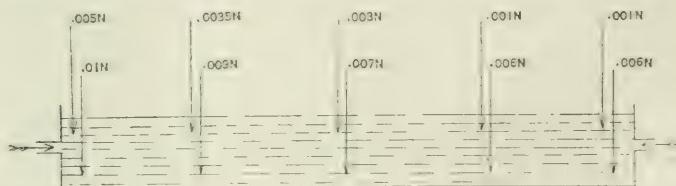


Fig. 1 Longitudinal section through the gradient tank. The figures indicate the concentrations of the salt at the depths indicated by the arrows. These concentrations were ascertained by determining the conductivity of samples taken from the different parts of the tank; in determining the gradient 7 samples along any given level were taken; only five are shown in the figure.

was made by means of the conductivity method. Figure 1 shows the gradient as it existed after the flows at the ends had been running for some time.

It will be noted from figure 1 that at any given level there is a gradient of salt from end to end of the tank. The concentration at the bottom of the tank was much higher than that near the surface of the water, and thus the fishes at times reacted to the vertical gradient, which was much sharper than the horizontal one. This reaction to the vertical gradient did not interfere greatly with the experiments, however, because the fishes tend to swim back and forth in the tank at whatever level they may be. Furthermore, most of the fishes worked with, remained near the bottom for a large proportion of the time. A further,

point brought out by the conductivity measurements was that the water, after flowing in at the ends of the tank for 15 minutes or less, often showed a piling up of the salt at a point about two-thirds of the way to the tap water end, i.e., a little past the middle. This piling up was brought out graphically by the use of colored salt solutions, which showed a more intense color at this point for a short time. Later the deepening in color disappeared, and tests showed the gradient to be continuous from one end of the tank to the other.

Before the fact of the piling up of the salt was discovered, it was noted that the fishes often gave a negative reaction to this part of the tank. With the demonstration of the increased salt concentration at the point in question, and the fact that the increase disappeared after the flow at the ends had been on for about 30 minutes, most of the experiments were delayed until sufficient time had elapsed for the adjustment to take place; any marked reaction of the fishes at the point of higher concentration, was noted and recorded. That the gradient as shown in figure 1 is a typical gradient is supported by the fact that Shelford and Powers ('15) figure a similar gradient which they obtained between sea-water and fresh water, in their work with marine fishes. In the following gradient experiments, attention should be called to the fact that the reactions whether positive or negative are seldom 100 per cent reactions. In other words, the fishes are nearly always positive to some concentration of the salt in question. It seems that for most fresh water fishes there exists an optimum salt concentration somewhere between a 0.01N and that of the tap water. This fact is brought out in the experiments with a majority of the salts.

The species of fishes used principally have been the black bullhead (*Ameiurus melas*), blue gills (*Lepomis pallidus*), rock bass (*Ambloplites rupestris*), green spotted sun fish (*Lepomis cyanellus*), white crappie (*Pomoxis annularis*), pumpkin seed (*Eupomotus gibbosus*), and small mouth black bass (*Micropterus dolomieu*). Numerous experiments have also been run with various species of Cyprinid minnows.

## IV. PRESENTATION OF DATA

## A. REACTION EXPERIMENTS

*I. Reaction to chlorides*

The fishes used are less sensitive to the chlorides of the salts than they are to the nitrates and sulphates. They also react differently in the presence of different chlorides. Thus they are sensitive to both the anions and the cations, and to different degrees.

*a. Ammonium chloride.* The fishes were decidedly negative to this salt in 0.01N concentration. The experiments were run in water that was a mixture of half aerated and half unaerated tap water (i.e., moderately acid with  $\text{CO}_2$ ). It has been found (Wells '15 a) that fishes give normal reactions in this water.

*b. Potassium chloride.* These experiments were also performed in water which was somewhat acid. The reaction of the fishes was rather peculiar in that they were positive to a higher concentration of this salt than was expected. Twenty-one experiments were performed and all showed this phenomenon. In a number of cases the fishes selected the highest concentration for a large part of the time. It was thought that the reaction might be due to the positiveness of the fishes for the chlorine ion, as will come out in other experiments; the known toxicity of the potassium ion, however, made this conclusion seem doubtful. Again, the fishes had been in the laboratory for over a month and were somewhat starved. It had already been determined that starvation increases the positiveness of some fishes to certain salts, and thus the reaction might be laid to this. However, the real explanation was later found to lie in a mutual antagonism which exists between certain salts and acids. Thus the reaction of the fishes in selecting the salt end was a reaction which brought them into the lesser stimulating part of the gradient. In the tap water end, the  $\text{CO}_2$  made the water quite acid. In the salt end this action of the acid was neutralized by the presence of the salt and vice versa. This phenomenon was noted in a number of the gradient experiments, while its cause was definitely proved in the resistance experiments.

*c. Sodium chloride.* This was the first salt to be experimented with at the University of Illinois and a large number of experiments (46 in all) was performed with it, as the reactions of the fishes were not what was at first expected. Experiments were run in aerated (neutral) water, in moderately acid water (8–10 cc. per liter) and in strongly acid water (18 cc.  $\text{CO}_2$  per liter).

It had been noted that the fishes became sluggish when kept in the aerated water, and because they reacted positively to the  $\text{NaCl}$  in the gradients in this neutral water, the experiments were repeated in acid water to make the results certain. The fishes were positive to the  $\text{NaCl}$  half of the tank in all three kinds of water, but were markedly most positive in the most acid water. They are negative to this water alone, because of its marked acidity. The increase in positiveness to the  $\text{NaCl}$  in the acid water must be due to the fact that the salt antagonizes the stimulating action of the acid and thus the fishes selected the portion of the tank where they were the least stimulated, as they did in the case of the  $\text{KCl}$  gradient in acid water.

In an attempt to drive the fishes out of the salt end, the  $\text{NaCl}$  concentration was increased to 0.02N but without diminishing the positive reaction. In the strongly acid water the fishes were found to give a positive reaction to as small a concentration of  $\text{NaCl}$  as 0.001N though the reaction to this low concentration was not so definite as with the higher concentrations. The reaction to  $\text{NaCl}$  varied somewhat with the species; the crappies and bull-heads were positive in all three kinds of water while the blue-gills were positive in the neutral and strongly acid water but were indifferent to negative, in the moderately acid water.

Ten experiments with 0.01N  $\text{NaCl}$ , in distilled water, were run to check those with the tap water. The results show the fishes to be markedly positive to the  $\text{NaCl}$  in distilled water gradients; this positiveness is not as great as in the acid water, but is great enough to show conclusively that the fishes used are positive to  $\text{NaCl}$  in concentrations very little lower than 0.01N.

*d. Calcium chloride.* Calcium chloride was the first salt used at Chicago in the gradient experiments. It was found that normal fishes (large rock bass are exceptions) are negative to

a 0.01N solution of this salt, and the graphs show this negativity to be rather definite. The fishes turned back from the  $\text{CaCl}_2$  end at a point which the conductivity measurements showed to be about 0.0065N. Some of the apparently normal fishes, however, gave positive reactions to 0.01N  $\text{CaCl}_2$  and in working out this point over 150 experiments were performed. A very interesting relation between starvation and the reaction of fishes to  $\text{CaCl}_2$ , and probably some other salts, was found to exist. The experiments showing this relation will be discussed on a subsequent page under the heading, "Physiological states and the reactions of fishes" (p. 260).

e. *Magnesium chloride.* Normal fishes reacted negatively to a 0.01N concentration of this salt, but as with calcium, there was a number of instances where the reaction seemed to be reversed. Normal fishes were also negative to a 0.02N concentration, which however did not prevent a few of the fishes from showing a positive reaction, as they had done with the 0.01N solution.

## 2. Reaction to the nitrates

The nitrate experiments, with the exception of part of those with calcium, were performed at Illinois. The experiments with the nitrate of calcium were performed largely at Chicago, enough being repeated at Illinois to correlate the reaction in the two waters.

a. *Ammonium nitrate.* Practically all the fishes used were negative to this nitrate, which is very stimulating to them, in tap water, as will be shown in the resistance experiments. They did not, however, avoid the salt end with as much precision as is displayed in the case of a number of the other salts, and in one experiment, a 25-gram crappie, although giving a fairly strong negative graph, still was overcome by the salt, lost control of its movements, and 'scooted' about the tank, finally leaping over the edge onto the water table. Sixteen experiments were performed: of these fourteen show decidedly negative reactions, while two, one with a 3-gram blue-gill and one with a 6-inch bull-head, show positive reactions. These two fishes were not

overcome by the salt, though they remained in the salt end during a majority of the 15 minutes that they were in the tank.

*b. Potassium nitrate.* The fishes were consistently negative to this salt in 0.01N concentration. Of 40 fishes tried in the gradient, 27 gave decidedly negative reactions, 5 stayed in the middle third of the tank, and 7 were more or less positive. In only 3 experiments was the time spent in the salt half of the tank, over 60 per cent of the total time. Of the 27 negative fishes, 20 spent over 80 per cent of the time in the tap water end.

*c. Sodium nitrate.* Experiments with all three kinds of water were run. In the neutral water the fishes were decidedly negative the graphs showing that 86 per cent of the time was spent in the tap water end of the tank. In the moderately acid water, 70 per cent of the reactions were negative and 30 per cent positive. In the strongly acid water, the fishes were decidedly positive to the 0.01N concentration showing an 81 per cent positive reaction. The concentration of the salt was now decreased to 0.002N and the same fishes tried. They were not so positive to this small concentration in the acid water as they had been to the 0.01N solution but they were still more positive than in the moderately acid water. The graphs show 45 per cent of the time was spent in the salt third of the tank, 30 per cent in the middle third, and 25 per cent in the tap water third. These results show again the effect upon the behavior of the fishes, of the antagonistic reaction between the acid and the salt; they select the higher concentration of salt in the gradient in strongly acid water but are negative to this same concentration in water which is not so acid. Note also (table 1, p. 256) that the antagonism between the salts and the acid seems to be more marked in the case of the K salts. Table 1 shows that in the case of both the chloride and nitrate of potassium the antagonism between the salt and the acid was sufficient to cause the fishes to react positively in moderately acid water. With sodium, the chloride shows a positive reaction in the moderately acid water but in this same water, the nitrate gives a negative reaction. It is not until the water has been made strongly acid that the fishes react positively to the nitrate of sodium.

*d. Calcium nitrate.* At Chicago 20 (40-min.) experiments were run with this salt. The reactions of the fishes were so decidedly negative that further work seemed unnecessary. At the University of Illinois, it was decided to repeat the experiments with calcium nitrate as a check upon the reactions of the fishes in the two waters. To this end a series of experiments with 0.01N  $\text{Ca}(\text{NO}_3)_2$  in neutral water, was run. The results were very different from those obtained at Chicago. There the fishes had shown a 90 per cent negative reaction to this salt in 0.01N concentration, while at Illinois in the neutral water, the reaction was 50 per cent negative and 50 per cent positive. In other words they seemed to be indifferent to the salt. It was thought that the explanation of the Illinois reaction might lie in the fact that, since calcium nitrate hydrolyzes to give a faintly acid solution, the fishes, which (Wells '15 a) had already been shown to be negative to the neutral water, were reacting to this acidity. This proved to be the case, for when the experiments were repeated in moderately acid water, the fishes gave an 80 per cent negative reaction.

To make doubly sure of the results with the calcium nitrate, a final series of experiments was run in distilled water, which it will be remembered is slightly acid with  $\text{CO}_2$  (2-3 cc. per liter). Five 15-minute graphing experiments were run with results that show a 75 per cent negative reaction. An experiment with 4 bull-heads (3-5 in. long) was read 50 times at 30-second intervals. Computation showed that the fishes had spent 74 per cent of the time in the negative half of the tank. Thus the reactions at Chicago and at Illinois, when slightly acid water is used, are in close agreement in showing the negative reaction of fishes to 0.01N concentration of calcium nitrate.

*e. Magnesium nitrate.* Twelve experiments were performed with this salt at Chicago; they showed a 100 per cent preference for the tap water half of the tank. The negativeness was more marked in some experiments than in others but in none did the fishes swim into the salt end. The experiments have not been repeated at Illinois.

### 3. Reaction to sulphates

a. *Ammonium sulphate.* Fishes are negative to this salt as to the other ammonium salts. All the ammonium salts are strongly toxic to the fishes used. Especially is this toxic reaction noticeable in the tap water. The explanation for this will be taken up in the discussion of the resistance of fishes to ammonium salts.

b. *Potassium sulphate.* Fishes are decidedly negative to 0.01N concentration of potassium sulphate. Twelve experiments were performed in moderately acid water and in none did the fishes give a positive reaction. In one the fish selected the middle third of the tank, but turned back regularly from the salt end. The results with this salt illustrate the increasing toxicity of the anion; it will be remembered that in moderately acid water the fishes gave a positive reaction to the nitrate of potassium. No experiments were performed at Chicago with this salt.

c. *Sodium sulphate.* No experiments were performed at Chicago with this salt. At Illinois two series were run, one in moderately acid water, and the other in strongly acid water. The reactions in the two kinds of water were very similar to those obtained with sodium nitrate in the same kinds of water. It was noted that in the strongly acid water the fishes often spent much of the time at the surface and were thus not swimming in the strongest gradient. For this reason the reactions might be expected to be somewhat less definite but the results show very little difference in cases where the fishes stayed at the bottom or swam at the surface. In 4 experiments the fishes spent practically all the time at the surface and 60 to 90 per cent in the salt half of the tank. In 8 experiments with this strongly acid water the fishes remained at the bottom throughout; seven of these experiments show a decided preference for the salt end while one was negative.

In the  $\text{NaNO}_3$  experiments, it will be remembered, the fishes were negative to the salt in moderately acid water and this was also found to be the case with the sulphate. Fifteen experi-

ments with 0.01N  $\text{Na}_2\text{SO}_4$  in moderately acid water were run. All of them show decidedly negative reactions. In a number of experiments the attempt was made to drive the fishes into the salt end, but with no success, except in one case, where a 20-gram crappie was driven into the salt end and remained there for 5 minutes before swimming back into the tap water end. The blue-gills could not be driven as they would dart back past the driving rod in every case. If these fishes were dropped into the salt end they showed much disturbance and very soon swam into the tap water end. In one experiment 8 small blue-gills (2-4 grams) were placed in the tank and readings of their position taken every 30 seconds until 25 readings had been made. The percentage of time spent in the thirds of the tank were, salt third 13 per cent, middle third 36 per cent and tap water third 51 per cent. Thus fishes are negative to 0.01N sodium sulphate in moderately acid tap water but are positive to this concentration in strongly acid water. The explanation of this latter reaction must lie in the antagonism between the salt and the acid.

*d. Calcium sulphate.* These experiments (11 in all) were performed at the University of Illinois. The reaction in moderately acid water was negative in all but two cases. In one of these a fish which had at first selected the tap water end, was driven into the salt end, where it remained for the remainder of the experiment. An experiment in which 10 small blue-gills (2-4 grams) were placed in the tank and their positions read at 30-second intervals, showed percentages as follows: Time spent in salt third 25 per cent, in middle third 32 per cent and in the tap water third 43 per cent.

*e. Magnesium sulphate.* Ten experiments with this salt were run at Chicago. All showed a negative reaction to the 0.01N concentration and in most cases the reaction was very decided (usually above 80 per cent in tap end). The experiments were not repeated at Illinois.

*4. Conclusions from reaction experiments*

We note from the data which have been given that fishes are markedly sensitive to salts in solution and that they react to them in a definite manner. They are negative to 0.01N concentrations of most of the salts used, if in water which is moderately acid; this is the normal condition in most natural bodies of water. When the water becomes strongly acid, the reactions of the fishes are modified and may be reversed by the mutual antagonism which exists between salts and acids. So far as these experiments show, this antagonism exists only between the salts of K and Na and carbonic acid. From the general work upon the antagonism of salts, to be discussed later, one would not expect the antagonism to extend to the salts of Ca and Mg. In the reaction experiments it was seen that the fishes are, in nearly all cases, positive to some concentration of the salt in question. This positiveness is most noticeable in the case of NaCl. Table 1 is introduced to summarize the reactions of the fishes to 0.01N salt concentrations in the different kinds of water used.

**B. ANTAGONIZING SALTS AND THE REACTIONS OF FISHES**

To determine whether or not fishes detect and react to combinations of salts in gradients, a number of experiments was performed, based upon the phenomena of the antagonistic reaction of salts, which are familiar to all biologists. These phenomena in their simplest form are expressed in the antagonism which exists between the salts of Na and K, on the one hand, and Ca and Mg, on the other. There has also been found a certain degree of antagonism between Ca and Mg in some cases (Meltzer and Auer '08). Most of the work on the antagonism of salts has been done either upon single organs as the heart, or other muscle tissue, or upon developing eggs and embryos. Certain combinations have also been shown to be best for preserving the life of fishes and other fresh water animals in distilled water (Ringer, etc.). So far as I am aware, no attempt has ever been made to determine the reactions of fishes to com-

TABLE 1

*Showing the reactions of fishes to .01N concentrations of various salts, in gradients of different kinds of water, i.e., waters of different degrees of acidity*

SALT	PER CENT POSITIVE OR NEGATIVE IN, A GRADIENT OF THE SALT IN, DIFFERENT KINDS OF WATER				
	Neutral tap water	Distilled water faintly acid	Moderately acid tap water	Strongly acid tap water	
Chlorides.....	+	-	+	-	+
Ammonium....	-	-	-	-	21 79
Potassium....	-	-	-	-	67 33
Sodium.....	63 37	-	65 35	-	76 24
Calcium.....	-	-	-	-	26 74
Magnesium....	-	-	-	-	21* 79*
Nitrates					
Ammonium....	-	-	-	-	35 65
Potassium....	30 70	-	-	-	69 31
Sodium.....	-	-	14 86	-	30 70
Calcium.....	50 50	-	21 79	-	20 80
Magnesium....	-	-	-	-	0* 100*
Sulphates					
Ammonium	-	-	-	-	30 70
Potassium....	-	-	-	-	0 100
Sodium.....	-	-	-	-	0 100
Calcium.....	-	-	-	-	15 85
Magnesium....	-	-	-	-	14* 86*

\* Work done at Chicago.

TABLE 2

*Comparing the reactions of fishes to single salts and to combinations of antagonistic salts. One salt is present in .01N concentration and the other as a trace (.0002N). Slightly acid tap contains 6-8 cc. CO<sub>2</sub> per liter; strongly acid 18 cc. CO<sub>2</sub> per liter; distilled water is faintly acid (2-3 cc. CO<sub>2</sub> per liter)*

SALT	KIND OF WATER USED IN GRADIENT	REACTION OF FISHES IN PER CENT OF TIME SPENT IN HALVES OF TANK. POSITIVE = IN SALT HALF; NEGATIVE = IN TAP OR DISTILLED WATER HALF	
		Percent positive	Percent negative
NaNO <sub>3</sub> alone.....	Slightly acid tap	30	70
NaNO <sub>3</sub> + trace Ca (NO <sub>3</sub> ) <sub>2</sub> .....	Slightly acid tap	79	21
NaNO <sub>3</sub> + trace Ca (NO <sub>3</sub> ) <sub>2</sub> .....	Strongly acid tap	96	4
Ca (NO <sub>3</sub> ) <sub>2</sub> alone.....	Slightly acid tap	23	77
Ca (NO <sub>3</sub> ) <sub>2</sub> + trace NaNO <sub>3</sub> .....	Slightly acid tap	76	14
Ca (NO <sub>3</sub> ) <sub>2</sub> alone.....	Distilled water	19	81
Ca (NO <sub>3</sub> ) <sub>2</sub> + trace NaNO <sub>3</sub> .....	Distilled water	87	13
Ca (NO <sub>3</sub> ) <sub>2</sub> + trace Mg(NO <sub>3</sub> ) <sub>2</sub> ...	Strongly acid tap	53	47

binations of salts in a gradient, and I present a number of experiments of this sort here. They show that fishes recognize and react to combinations of salts according to the prediction which might have been made from the results of previous work upon antagonism.

It will be remembered that fishes are slightly positive to 0.01N NaNO<sub>3</sub> in moderately acid water. It was found, however, that they are negative to this salt in water that is but faintly acid and for this reason the following experiments were run in water which contained less than 8 cc. CO<sub>2</sub> per liter. The experiments were first run as regular salt experiments such as have been described before, and then the antagonizing salt was added to the salt flow. The concentration of the original salt was always 0.01N and that of the antagonizing salt 0.0002N, i.e., a bare trace was added. The results of these experiments are shown in table 2. A considerable number of experiments was run with each combination in the tap water, and then some check experiments were run in distilled water. The results in the distilled water gradients are very similar to those in the tap water.

#### *Discussion of the experiments with antagonizing salt combinations*

Table 2 shows clearly that the antagonistic action of the salts is detected and reacted to by the fishes. This is shown, for instance, in the sodium nitrate experiments; here the fishes were 70 per cent negative to this salt in slightly acid water but when a trace of calcium nitrate was added the negative response fell off to 21 per cent and the positive rose from 30 to 79 per cent. Then in strongly acid water the positive response increased to 96 per cent. The reactions of fishes in any gradient are due to their tendency to move about until they reach an environment that neither over- nor under-stimulates them. Thus they will not remain quietly in water that is strongly acid nor will they do so in water that is neutral. A slight degree of acidity (1-6 cc. CO<sub>2</sub> per liter, Wells '15 a) furnishes their optimum stimulation as far as H ion is concerned. The reversal in reaction of the fishes in gradients to which a trace of an antagonistic salt

has been added, must then be due to the fact that this trace of salt lessens the stimulation in the salt end of the gradient. There are three principal factors affecting the degree of stimulation of the gradients referred to in table 2, namely, the original salt (e.g.,  $\text{NaNO}_3$ ) the antagonising salt (e.g.,  $\text{Ca}(\text{NO}_3)_2$ ), and the acid. Before the antagonising salt was added the fishes were negative to the original salt, even though this meant spending most of the time in a degree of acidity which was slightly above their optimum. With the addition of the antagonising salt, however, they reversed their reaction and became positive to the salt end. The antagonising salt must have diminished the original stimulation in the salt end or have increased the stimulation in the acid end, or both. The work upon the effect of acids and salts upon permeability suggests that both factors were concerned. Lillie ('10) has shown that calcium salts decrease the permeability of egg membranes while the salts of sodium increase the permeability. Osterhout ('12, a and b) has shown that sodium salts increase the permeability of plant cells while the addition of a trace of calcium salt maintains normal permeability even in the presence of an excess of the sodium salt. Osterhout has also shown that there exists a mutual antagonism between certain acids and salts as for instance between  $\text{HCl}$  and  $\text{NaCl}$ , but the salts of calcium and magnesium work with rather than against the acid.

In the above experiment, therefore, the addition of the calcium salt to the end of the gradient which contained a sodium salt in concentration strong enough to cause the fishes to give a negative reaction, resulted in the fishes becoming positive. This reversal in the reaction of the fishes must have been due to the decrease in the stimulating power of the salt end. It has already been shown that increasing the acidity of water will cause fishes to become positive to concentrations of sodium salts to which they are normally negative (table 2) and it was found that the higher the acidity the higher was the concentration of sodium salt selected by the fishes.

Table 2 also shows that the antagonistic action between calcium and sodium salts is detected and reacted to, when the con-

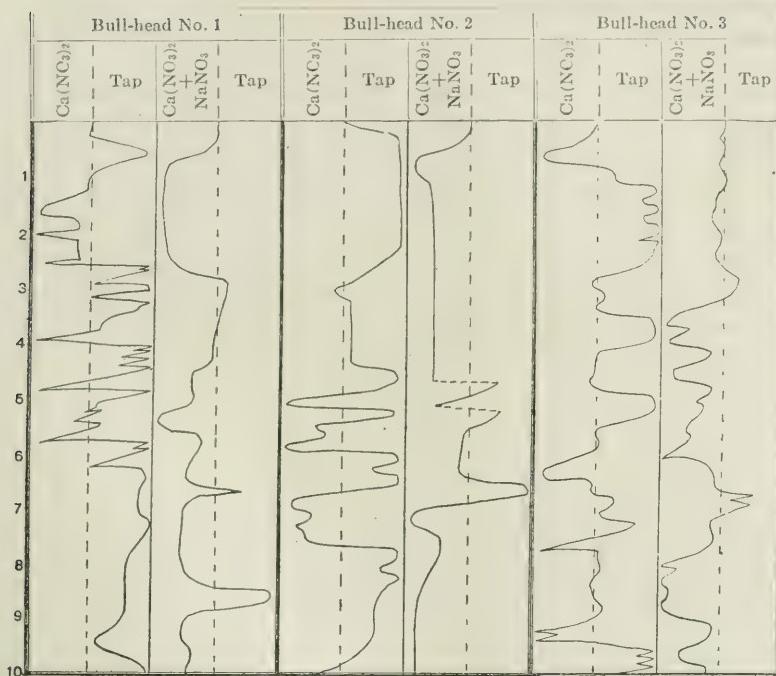


Fig. 2 Showing the reaction of bull-heads (*Ameiurus melas*) to  $\text{Ca}(\text{NO}_3)_2$ , alone, and in combination with a trace of  $\text{NaNO}_3$ . The concentration of the calcium salt was 0.01 N throughout and that of the sodium salt 0.0002 N; experiments performed in distilled water; numbers at left indicate time in minutes.

centrations of the two salts are reversed, i.e., when the calcium nitrate is present in 0.01N concentration and the sodium as a trace (0.0002N). The data for table 2 were obtained from graph experiments and also from readings. The same fishes were used in the gradient with the different conditions, i.e., they were first graphed in the gradient with the sodium salt (e.g.) alone and then again after the calcium salt had been added to the flow. To illustrate more accurately this method of experiment figure 2 is inserted. The graphs shown in this figure are 3 of those made by 4 bull-heads. The experiments were run as follows: The gradient with only  $\text{Ca}(\text{NO}_3)_2$  flowing in at the salt end, was obtained by allowing the flow to continue for 30

minutes. The fishes were then taken from the large aquarium and placed in pans of water, numbered 1, etc. The fish from pan No. 1 was placed in the gradient and its movements graphed for 15 minutes. It was removed and No. 2 was placed in the gradient and graphed. This was repeated for Nos. 3 and 4. A trace of  $\text{NaNO}_3$  was now added to the inflow at the salt end; after 20 minutes fish No. 1 was again placed in the gradient and its movements graphed for 15 minutes. This was repeated for the three remaining fishes in the same order as before. The graphs show the marked difference in the reactions of the fishes before and after the trace of sodium nitrate was added.

#### C. PHYSIOLOGICAL STATES AND THE REACTIONS OF FISHES

In the discussion so far attention has been called to the fact that in most of the series of experiments, there was a small percentage of the fishes (usually 3-5 per cent) which gave reactions more or less the reverse of those given by the majority. Such exceptions to the general behavior are common in experimental work of all sorts and probably indicate physiological differences upon the part of the organisms. That such physiological differences, i.e., physiological states, exist and that they influence very markedly the reactions of the animals has been proven beyond doubt (Child '13, and Allee '12). Allee and Tashiro ('14) have shown that the reactions of isopods are very closely correlated with the metabolic activity and Allee ('12) has shown that by changing the rate of metabolism he can alter and even reverse the reaction of isopods to current. A correlation between the rate of metabolism and the reactions of amphipods has been shown by Phipps ('15).

At Chicago during the winter of 1913-1914, a study not yet published was being made of the effect of starvation upon the resistance of fishes to KCN and low oxygen; it was thought that the starving fishes furnished good material for ascertaining during the same period something of the effects of starvation upon the reactions of fishes in gradients. Accordingly a series of 89 experiments was run with the starving fishes in gradients; 50

of the experiments were in gradients of  $\text{CaCl}_2$  since it seemed best to confine the experiments to a few salts at the most. It was decided that the starving fishes should not be handled to any great extent during the obtaining of the data for which the material was originally intended. A few experiments were run in gradients of  $\text{Ca}(\text{NO}_3)_2$  and  $\text{MgCl}_2$  the results of which were much like those for  $\text{CaCl}_2$ . Nine experiments with starving fishes in low oxygen gradients are included as they are significant.

The experiments with starvation and resistance of fishes showed in brief the following points: The fishes as they began to starve became more resistant to KCN and low oxygen. This rise in resistance which is a decrease in susceptibility, continued for some weeks (varied with species). There was then a rather sudden decrease in resistance (increase in susceptibility) which was found to be a close fore-runner of death. In terms of metabolism, as starvation in certain fishes proceeds the rate of metabolic activity is at first decreased. After remaining below normal for some weeks (or even months) the forces which are inhibiting the rate of reaction, give way and the rate runs up rapidly to, and beyond, the normal rate. Whether the changes in the physiological condition of the fishes are wholly quantitative is not certain. It is very probable that a change in the *rate* of metabolism does not express all that takes place but there may be alterations in the kind of metabolism also; in other words starvation in fishes may produce qualitative as well as quantitative changes in metabolism.

Starvation experiments were run with several species of fishes including the rock bass (*Ambloplites rupestris*), small mouth black bass (*Micropterus dolomieu*), pumpkin seed (*Eupomotus gibbosus*), mud minnow (*Umbra limi*), and the black bull-head (*Ameiurus melas*). The fishes seemed to be divided into two groups as far as their starvation reactions are concerned. The bull-heads made up one group and the other fishes a second. Most of the work was done with the bull-heads and the rock bass as representatives of the two groups. In the case of the rock bass some quantitative data can be presented.

1. *Reactions of starved fishes to CaCl<sub>2</sub>*

Normal bull-heads are negative to 0.01N calcium chloride in a gradient. It was noticed, however, that when food was given these fishes they often became positive to the salt half of the tank. To check this reaction 23 experiments with normal, well fed and starved bull-heads were run. Table 3 shows the results obtained. It shows that normal fishes (bull-heads) are negative to 0.01N calcium chloride well-fed ones positive, and starved negative again. The well-fed bull-heads were in fact given all the food they would eat and thus were really over fed, as they ate until their abdomens were much puffed out. The data in

TABLE 3

*Showing the reactions of normal, over-fed, and starved bull-heads (*Ameiurus melas*) to .01N calcium chloride, in a gradient. Data shows per cent of time spent in the halves of the tank*

FISH NUMBER	NORMAL REACTION		OVER-FED REACTION		STARVED REACTION	
	CaCl <sub>2</sub>	Tap	CaCl <sub>2</sub>	Tap	CaCl <sub>2</sub>	Tap
1	29	71	66.5	33.5	32	68
2	44	56	78	22	63	37
3	34	66	57	43	29	71
4	37	63	73	27	40	60
5	31	69	78	22	52	48

table 3 is taken from the graphs made with 5 fishes. The normal reaction of each fish was determined immediately upon bringing it into the laboratory from the streams. On the next day the fishes were fed all the beef they would eat and graphed again on the third day. They were then starved and graphed from day to day. The figures in column 4, table 2, are those obtained after from 5 to 10 days starving. Each day calcium chloride was run into the end of the tank opposite that of the day before.

The method of experimenting with the rock bass in the resistance experiments was to bring them in from the creeks in which they live and to weigh them individually, and at once. The process of starvation was then kept track of by successive weighings. Twenty-six experiments with these starving fishes were

run to determine the effect of the starvation upon the reaction to  $\text{CaCl}_2$ . It will be recalled that normal rock bass are negative to this salt in 0.01N concentration (except in the case of large fishes). The starving rock bass were therefore experimented upon in gradients of  $\text{CaCl}_2$  at different stages of starvation, with results such as those shown in table 4. This table shows that starvation increases the percent of positiveness of these fishes. This is true for that period of starvation, during which the rate of metabolism is slowed up. The few experiments that were performed upon fishes in which the factors inhibiting starvation had broken down and the rate of metabolism had gone above normal, indicate that the fishes are again negative to Ca salts at this time.

TABLE 4

*Showing the reactions of normal and starved rock bass (*Ambloplites rupestris*) to .01N concentrations of  $\text{CaCl}_2$  in a gradient. Reactions are shown in per cent of time spent in the two halves of the gradient tank*

FISH NUMBER	DATE OF COLLECTION	DATE OF EXPERIMENT	ORIGINAL WEIGHT IN GRAMS	WEIGHT AT TIME OF EXPERIMENT	REACTION IN PER CENT OF TIME IN	
					$\text{CaCl}_2$	Tap water
1	Nov. 20	Nov. 23	9.9	8.9	30	70
2	20	23	23.1	22.5	43	57
3	20	23	56.2	54.5	38	62
4	20	23	70.6	68.4	22	78
5	20	23	126.0	124.0	90	10
6	Oct. 16	23	21.1	18.6	100	0
7	16	23	66.0	61.7	30	70
8	16	23	90.9	77.0	10	90
		1914				
9	Dec. 6	April 9	97.0	64.2	38	62
9	6	10	97.0	64.0	73	27
9	6	10	ends of gradient reversed		82	18
9	6	15	97.0	67.3	34	66
9	6	15	ends of gradient reversed		30	70
9	6	16	97.0	65.0	67	33
9	6	17	97.0	62.3	76	24
10	6	5	83	64.2	46	54
10	6	10	83	61.7	61	39
10	6	10	ends of gradient reversed		58	42
10	6	16	83	61.0	60	40

Note (table 4) that the normal fishes were negative to 0.01N CaCl<sub>2</sub>, that with the small fishes this reaction had become positive by the end of a little over a month (fish No. 6) while the larger fishes were still negative. Fishes Nos. 9 and 10 show the reaction of fishes starved for almost four months. These fishes were kept in running water and probably obtained a little food but the successive weighings showed that the process of starvation was a continuous one. Note the reversal in reaction of fish No. 9. The first experiment with this fish shows it to be slightly negative. On the next day it had become positive, as was shown by two experiments, with the salt flow at one end of the gradient tank in one, and reversed in the other. The weighings show that the fish had increased in weight since the day before and this increase must have been due to the securing of food in some way; the food had temporarily restored the normal reaction. However, by the next day the weight had again fallen off and the fish was once more positive to the salt, as is characteristic for starving fishes.

## 2. Reaction of starved fishes to low oxygen

The results of the experiments with starved fishes (rock bass) in low oxygen gradients are seen in table 5, which shows that

TABLE 5

*Showing the reactions of normal and starved rock bass to low oxygen in a gradient. Reactions are expressed in per cent of time in the halves of the tank (work done at Chicago)*

FISH NUMBER	DATE OF COLLECTION	DATE OF EXPERIMENT	ORIGINAL WEIGHT	WT. AT AT TIME OF EXPT.	REACTIONS IN PERCENT OF TIME IN	
					Low O <sub>2</sub>	Tap water
Normal fishes						
1	Nov. 20	Nov. 22	1.7	1.5	5	95
2	20	22	9.9	9.5	25	75
3	20	22	23.1	22.5	32	68
4	20	22	70.6	69.0	20	80
5	20	22	126.0	124.0	91	9
Starved fishes						
6	Oct. 16	22	21.1	18.6	44	56
7	16	22	66.0	61.7	50	50
8	16	22	90.9	77.0	34	66

normal rock bass are negative to low oxygen (1 cc. per liter at the low end) as has been shown also by Shelford and Allee ('13, p. 236). Large rock bass seem to be an exception to this general rule as they are not always negative to low oxygen, and in some cases seem to definitely prefer the low oxygen end of the gradient, spending a majority of the time there. The cause of this reaction has not been determined but it may have to do with the concentration of hydrogen ion which would probably be a little higher in the low oxygen end than in the high oxygen water, the difference being due to the difference in the effect of the two kinds of water upon the elimination of carbon dioxide by the organism.

Fishes Nos. 6 to 8 (table 5) are the individuals occurring under the same numbers in table 4. There it was noted that their reaction to the  $\text{CaCl}_2$  had become more positive than the normal reaction and in table 5 it will be noted that these fishes are less sensitive to the low oxygen also. Fish No. 5 is also the same in tables 4 and 5 and it will be noted that this fish was positive to both low oxygen and 0.01N  $\text{CaCl}_2$ . Experiments in low oxygen gradients were not performed with these fishes later in their period of starvation but the data given indicate that as they become somewhat starved they at the same time become less negative to low oxygen. This indicates that their metabolic rate is slower than normal.

#### D. ACCLIMATIZATION AND THE REACTION OF FISHES

During the course of the experiments, considerable evidence was accumulated concerning the effect of acclimatization upon the reactions of the fishes. A few experiments with fishes in  $\text{CO}_2$  gradients indicated that these fishes after living for two to three weeks in water whose  $\text{CO}_2$  concentration was 8 to 10 cc. per liter, were more sensitive to the  $\text{CO}_2$  than normal fishes. To determine whether or not the presence of an excess of salt would result in similar reactions to the salt in a gradient, a series of acclimatization experiments with  $\text{CaCl}_2$  was run.

A medium-sized (45-gram) rock bass was graphed in a  $\text{CaCl}_2$  gradient; its normal reaction was decidedly negative to 0.01N

$\text{CaCl}_2$ . It was now placed in a 20-gallon-jar full of a 0.01N solution of this salt. Each succeeding day it was taken from the jar and its reaction in the gradient graphed, when it was returned to the jar. This was continued for 6 days; the concentration of the solution in the jar was then raised to 0.05N. The fish was left in this solution 4 days longer, being graphed each day. It was then returned to the tap water and graphed again after 2 days. In making the graphs each day, the salt solution was run into the end of the gradient tank, opposite that of the day before. A series of the graphs made by this fish are shown in figure 3. They show the different stages in the process of acclimatization. In short they indicate that the

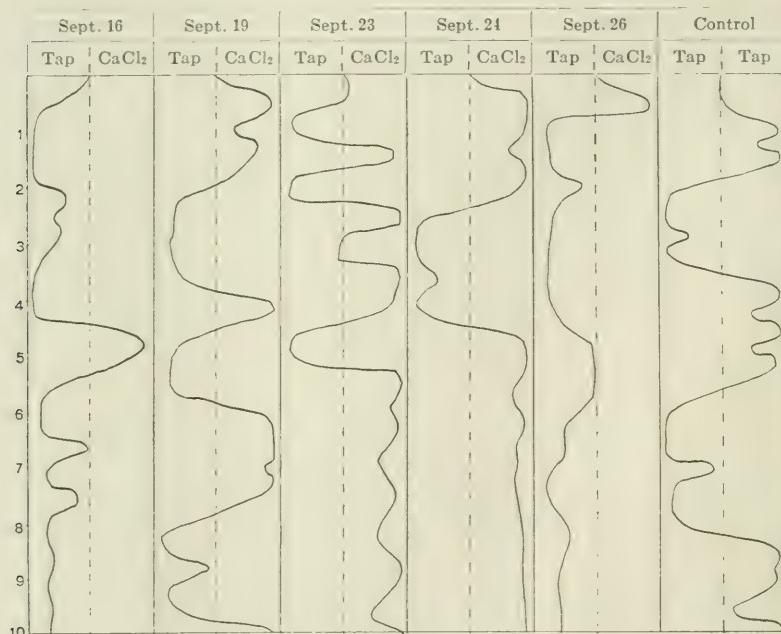


Fig. 3 Showing the reversal in reaction to 0.01 N  $\text{CaCl}_2$ , upon the part of a 45-gram rock bass (*Ambloplites rupestris*), after being kept in the salt solution for a week, and the return to normal reaction upon being placed in tap water again. In the experiments the salt was made to flow into alternate ends, but in the chart the graphs have been copied so that the reaction will be more easily seen, by keeping the same relation between the tap and  $\text{CaCl}_2$  ends.

fish did become acclimated to the  $\text{CaCl}_2$  solution by the end of a week and selected the higher concentration in the gradient. Then after 2 days in the tap water it was negative to the salt again.

A like set of experiments was performed with a small bull-head (6 in. long) with similar results; the acclimatization, however, came sooner. Neither of the fishes was fed while in the  $\text{CaCl}_2$  and this would have an effect upon their reaction. The fact that the rock bass became negative again after being returned to the tap water indicates that the starvation did not account for its positive reaction while being kept in the  $\text{CaCl}_2$  solution. Starvation would tend to increase the negativeness of bull-heads to the salt so the positive reaction upon being kept in the  $\text{CaCl}_2$  can be due to nothing but acclimatization. The difference in the effect of starvation upon the reactions of the two species of fishes to salts is probably due to a difference in the metabolism of the fishes and will be discussed in another paper.

#### E. RESISTANCE OF FISHES TO SALTS

The toxic effect of certain salts upon organisms has been the subject for considerable investigation upon the part of other workers (Ringer, Loeb, R. Lillie, and others) and therefore considerable is known concerning the relative toxicity of the various salt ions. In the present paper are presented data which indicate that much of the work upon the toxicity of salts must be reconsidered and correlated with the chemical reaction of the water. The data show that the poisonous properties of a given salt may vary within wide limits depending upon the amounts of hydrogen or hydroxyl ions present in the solution.

##### *1. Resistance to ammonium salts*

According to Mathews ('07) the pharmacological action of most salts is due to the ions of the salt. The kind of action depends upon the character of the charge of the ion, i.e., whether positive or negative; the degree of action is proportional to the available energy in the ion. Ammonia salts are peculiar, how-

ever, in that their toxicity is not due to the action of either of the original ions, but to the products which are derived from the breaking down of the original ammonia compound. Ammonia salts in solution dissociate principally into  $\text{NH}_4^+$  ions and the acid ion with which the ammonia is combined. There is a hydrolytic dissociation also, so that there is always present in the solution a small amount of the free acid and the ammonium hydrate. In considering the reactions of fishes to ammonium hydrate (Wells, '15a, p. 236) it was pointed out that the ammonium hydrate in solution is in equilibrium with and is but a small per cent of the dissolved ammonia gas. In the case of an ammonia salt the hydrolytic dissociation of the salt produces the hydrate, which in turn dissociates to give water and ammonia gas. The amount to which the salts dissociates into ammonium hydrate and ammonia gas varies with the salt, being least in the sulphate and larger in the carbonate (Mathews, *l.c.*). Mathews further states that it is probable that the action of the ammonium salts is due, therefore, to the hydrate which is formed, and in turn the action of the hydrate is dependent upon the action of the dissociated  $\text{NH}_3$ . This gas is probably in a nascent condition just at its moment of origin, when the valencies of the nitrogen are still open.

The toxic action of the ammonium salts used in the reaction experiments was found to be very marked when they were dissolved in the tap water, but was much less when the salts were dissolved in distilled water. Solutions (0.01N) of the chloride, nitrate and sulphate were made up in tap and distilled water and small blue-gills (3-gram) were placed in jars of the different solutions. The temperature was kept constant by setting the jars in running tap water. One liter of solution was contained in each; the results are shown in table 6.

The marked increase in the longevity of the fishes in the distilled water seemed worthy of further investigation. Death in the distilled water was in part due to increasing acidity of the solution, as titrations showed a concentration of hydrogen ion at the end of the experiments that must soon have killed the fishes even though no other factor were present. This increase

TABLE 6

*Showing the resistance of small blue-gills (3-gram) to .01N concentrations of the chlolide, nitrate and sulphate of ammonium dissolved in tap and distilled water*

KIND OF WATER	DYING TIME IN THE SOLUTIONS		
	Chloride	Nitrate	Sulphate
Tap water from aquarium.....	4.8 hours	3.9 hours	3.5 hours
Distilled water.....	18 days	16 days	17 days

in the acidity of the solutions was marked in the case of all three salts and the titrations showed that the acidity upon the day of death of the fishes, had increased to nearly 0.001N while 0.0001N is enough to kill these fishes in distilled water when no salt is present. The increase in acidity was not due entirely to the CO<sub>2</sub> given off by the fishes, as boiling did not remove it. It must, therefore, have come from the acid which had formed from the hydrolysis of the salt. The ammonia formed in the same process had passed off into the atmosphere. It seems clear then that the three salts in question do not furnish a large enough quantity of NH<sub>3</sub> to kill the fishes, if the salts are dissolved in distilled water.

It has been pointed out in a previous paper (Wells '15 a) that the tap water at the University of Illinois contains an unusually large amount of the bicarbonates of Ca and Mg and that as the water is aerated these bicarbonates dissociate to give the normal carbonate. It was thought that the toxicity of the ammonium salts in the tap water may have been due to the formation of ammonium carbonate and the further dissociation of this salt to give NH<sub>3</sub> in toxic quantities. To test this possibility three experiments with 0.01N concentrations of (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> in distilled water, were tried. The dry salt gave a strong odor of ammonia but the solution was too dilute to give any odor at all. After thoroughly shaking the solution and allowing it to stand for 10 minutes, a 10-gram sun-fish was placed in a liter of it. A control was run in distilled water. The result of this experiment, together with those obtained from a number of other experiments are given in table 7. The table shows that am-

monium carbonate is very toxic in distilled water, that standing does not lessen its toxicity greatly nor does it that of the sulphate in tap water, and finally that the ammonium sulphate is no longer toxic in tap water when the carbonates have been converted into sulphates by the addition of enough sulphuric acid to make the water neutral to methyl orange.

TABLE 7

*Showing the resistance of fishes to .01N concentrations of  $(NH_4)_2CO_3$  in distilled water; the effect of standing upon the toxicity of ammonium salt solutions; and the non toxicity of a solution of  $(NH_4)_2SO_4$  in tap water when the carbonates have been converted into sulphates.*

SOLUTION	DYING TIME OF THE FISH IN THE	
	Experiment	Control
0.01N $(NH_4)_2CO_3$ in distilled H <sub>2</sub> O.....	1.7 hours	normal
Same after standing 24 hrs.....	2.2 hours	normal
0.01N $(NH_4)_2SO_4$ in tap water.....	1.3 hours	normal
Similar solution after 24 hrs.....	2.2 hours	normal
0.01N $(NH_4)_2SO_4$ in tap water after the carbonates have been changed to sul- phates.....	fish normal at end of a month	control in .01N sulphate in or- dinary tap; dead in 2.1 hrs.

The experiments upon the resistance of fishes to ammonium salts show clearly that ammonia in any form is toxic to fishes in water containing carbonates. Since practically all natural waters contain a greater or lesser amount of the carbonates in solution as such, or as bicarbonates, the introduction of even very small amounts of ammonia into these waters will be very detrimental to the fishes. Table 7 shows, on the other hand, that the carbonates are not necessary to the immediate existence of the fishes, i.e., the water need not be alkaline to methyl orange as Marsh ('07) claimed. It may of course be that the carbonates are necessary to a successful completion of the life history of some fishes, or to the continued existence of certain species. This point has not been worked out so far as I am aware.

## 2. Resistance to potassium salts

Solutions (0.01N) of the chloride, nitrate, and sulphate, were made up in tap water and a small blue gill (3-5 gram) introduced into a liter of each; the results are shown in table 8.

The action of the potassium salts in tap water was checked by placing a fish in a 0.01N solution of the most toxic one, i.e., the sulphate, in distilled water. The reactions of this fish were very peculiar. After 3 days in the solution it was noticed that the fish was losing its equilibrium and it was expected that it would die in a few hours. On the next day, however, it was still

TABLE 8

*Showing the resistance of small blue gills to .01N concentrations of potassium salts in solution in tap water*

DYING TIME IN		
Chloride	Nitrate	Sulphate
Normal on 15th day.....	15 days	4 days

alive and for 10 days more it lived spending much of the time lying on its side but righting itself when touched with a glass rod. Its movements were sluggish and stiff, much as though it were dying from fungus disease. In all, the fish lived for 14 days in 0.01N potassium sulphate solution, which is over three times as long as a fish of the same size lived in the same strength solution in tap water. The long-drawn-out death of the fish is not a phenomenon that is peculiar to potassium salts, however, for it was noted that another small blue gill which was in an ammonium nitrate experiment in distilled water at the same time, gave a similar reaction. This latter fish swam about for three days on its side with the body bent into the bow-shape that often distorts fish after death, especially when they dry out. This suggests that the distortion may have been due to osmotic changes in the tissues.

### *3. Resistance to sodium salts*

Experiments with the following sodium salts were performed in tap water: bicarbonate, carbonate, chloride, nitrate, and sulphate. The solutions were 0.01N and the fishes small blue gills (3-5 grams). The results were as follows:

<i>Salt used</i>	<i>Resistance of fishes</i>
Sodium bicarbonate	Normal at end of 15 days; discont.
Sodium carbonate	Dead after 3 days
Sodium chloride	Normal after 19 days; discont.
Sodium nitrate	Dead after 31 days; only 50 cc. water left
Sodium sulphate	Normal after 20 days; discont.

From the above results we see that the sodium salts are not toxic to blue gills when 0.01N concentrations are used in tap water. The carbonate is an exception as the fish dies in this solution in 3 days. It has already been shown (Wells '15 a) that these fishes cannot live in water that is even faintly alkaline and thus the action of the carbonate is due to its alkalinity.

It will be remembered that the reactions of the fishes in salt gradients were complicated by the antagonism between the salts and the acid in the water. Loeb was the first to demonstrate that there exists an antagonism between salts and acids, as in 1899 he showed that acid antagonises the effect of NaCl on the swelling of muscle. He suggested that the antagonism depends upon the action of the substances upon the proteins of the tissues. Again, Loeb and Wasteneys ('11 and '12) demonstrated the antagonism between salts and acids, in their effect upon the marine fish Fundulus and explained the effect as due to a direct action on permeability. Osterhout ('14) made investigations which show that similar though less striking antagonism between acids and NaCl occurs in plants; he further states that the antagonism is not as great as that between NaCl and CaCl<sub>2</sub>.

To determine the relation of the antagonism between salts and acids to the resistance of fresh water fishes, a series of experiments was run with NaCl and HCl. Table 9 summarizes the results of these experiments. From this table it will be noted that fresh water fishes of the same species and size live much longer in toxic

TABLE 9

*Showing the antagonism of NaCl and HCl in their toxic action upon fishes; experiments performed in distilled water (U. of I.).*

SIZE AND SPECIES OF FISH	KIND OF SOLUTION	DYING TIME IN HOURS
25-gram rock bass.....	0.25N NaCl	18
23-gram rock bass.....	0.25N NaCl + 0.00005N HCl	41
12- gram green spotted sun-fish..	0.25N NaCl	48
8- gram green spotted sun-fish..	0.25N NaCl + 0.00005N HCl	144
3-gram green spotted sun-fish..	0.0001N HCl	48
3-gram green spotted sun-fish..	0.0001N HCl + 0.12N NaCl	normal at end of month
45-gram green spotted sun-fish..	0.25N NaCl + KOH to make just alk.	14

concentrations of NaCl when a trace of HCl is added. Also that fishes in toxic concentrations of HCl live longer when NaCl is present. Furthermore, NaCl is much more toxic in faintly alkaline solutions than it is in faintly acid solutions. All this agrees with Osterhout's conclusions as to the effect of alkalies and acids on permeability.

#### 4. Resistance to the salts of Ca and Mg

The only resistance experiments which have been carried on with these salts are some that were performed at Chicago. The experiments with Ca were performed in connection with the acclimatization experiments already discussed. In brief, it was found that the sun-fishes lived very well in 0.01N  $\text{CaCl}_2$ , while the bull-heads did not live so well. Other experiments showed this same relation for the nitrate and sulphate but the latter salts were decidedly more toxic than the chloride and the sun-fishes did not live well in solutions of them. An interesting fact was noted in connection with the  $\text{CaCl}_2$  experiments. A medium sized (50-gram) rock bass, after a week in 0.01N solution, showed signs of degeneration of the rays of the tail fin. This degeneration continued until nothing but the blood-reddened stub of the tail was left. The other fins were not affected; the tail fin regenerated when the fish was returned to tap water.

Day ('87, p. 203) states that in a certain lake in the British Isles, there is a race of tailless trout which some authors claim can be traced as due to the action of deleterious matter in the water. Day (*loc. cit.*) also quotes J. Harvie-Brown as saying, about 1876, "that a contraction of the rays of the tail fins of the trout in the river Carron occurred, and was believed to be due to the continuous pollution of the water through the agency of paper mills." Upon looking up the composition of the waste from the paper mills (Griffin and Little '94, and Phelps '09) I find that among other substances calcium is always present in large quantities, both as the chloride and in other combinations. Therefore the phenomenon reported by Day was likely due to the presence of an excess of calcium in the water.

Marsh ('07) has shown that the waste from paper mills is very toxic to fishes. Calcium is not especially important, however, as the toxicity of the waste is probably due to the excess of acidity or alkalinity, and perhaps to other toxic substances.

#### V. GENERAL DISCUSSION

The experiments discussed in the preceding pages will be considered very briefly in one or two phases of their general bearing. From an ecological point of view they emphasize once more the ability of fishes to recognize and react to environmental factors in very small concentration. It should be pointed out, however, that the reactions of fishes to salts in solution are by no means so delicate as their reactions to acids and alkalies, i.e., to hydrogen and hydroxyl ions. As a matter of fact the reaction to salts is complicated by the acid factor in many cases, as, for instance, when the salt gives an acid solution, but more especially in the numerous instances where there exists an antagonism between the salt and the acid. Thus fishes may react differently to a given salt concentration in water which is strongly acid and water that is but faintly acid. The resistance experiments show, also, that fishes can live in the presence of an acid concentration which would ordinarily kill them, if the proper concentration of the right salts is present. The work of Osterhout ('15) and others, as well as data presented in this paper,

indicates that the antagonism between the salts of calcium and magnesium is not nearly so marked as it is in the case of the salts of sodium and potassium. Since the former salts are by far the most common and plentiful in natural fresh waters, the importance of salts in nature in antagonising introduced acids is less than it would be were the salts of sodium and potassium plentiful. The problem is one which will furnish material for some very interesting ecological investigation.

The importance of small amounts of ammonia in natural waters has been pointed out in the discussion of these salts. The effects of starvation upon fish metabolism and reaction will be further discussed in another paper. There is an interesting possibility brought out by the acclimatization and other data, especially those pertaining to the importance of acids, that will be discussed here. This possibility relates to the movements of organisms in general but the present discussion will be limited to the very interesting migrations of the anadromous fishes.

The stimulus that causes anadromous fishes to spend part of their life cycle in fresh and part in salt water has long been a matter for speculation. Such stimulus must be related to the rhythmical metabolism of the animal, for it brings the fishes into the sea or fresh water at certain definite stages in the life cycle. The state of the metabolism of these fishes while they are in the fresh water, must differ very decidedly from that during the period of the life cycle which they spend in the ocean, for these two environments differ in two very important particulars, namely, the fresh water has a low specific gravity and is consistently acid in reaction, while the sea water has a relatively high specific gravity and is consistently alkaline. Also the reactions of the fishes are markedly different. In the fresh water they are positive to current, and, in a gradient, select water that is just on the acid side of neutrality and of lower density than that of the sea. Salt water fishes, on the other hand, are probably negative to a fresh water current, select water on the alkaline side of neutrality and reject water of low specific gravity for that of higher (Shelford and Powers '15). The reactions of the fishes in fresh water,

therefore, are the reverse of those in sea-water with regard to these three factors, and in the normal life cycle of such anadromous fishes as the salmon, this reversal in reaction must occur at least twice, once when the fishes leave the fresh water streams for the ocean, and again when they return. With species of salmon that breed more than once, the reversal must occur more often.

There are two general complexes of factors to be considered in an attempted explanation of the reactions of the anadromous fishes, namely, the fish and the environment. Both are made up of physico-chemical factors which are measurable, and to a large degree quantitatively. Of the two complexes, that of the living organism is least understood and perhaps, because it is much more variable and changing than the environmental complex, which, especially in the case of the sea-water, varies hardly at all. For the fishes to live normally in the environment there must exist between the two complexes a more or less complete equilibrium. A disturbance of this equilibrium resulting from a change in either of the complexes, will, if great enough or long enough continued, result in the death of the fishes unless by their reactions they seek out another environment which allows their physiological processes to proceed normally. It should be emphasized that the only mode of readjustment is through the proper reaction, either physiological or motile upon the part of the fishes, since the environment is much the more stable complex, and there is a great deal of evidence to show that of the two possible reactions upon the part of the living organism, the motile reaction is much more likely to occur than the physiological readjustment, i.e., acclimatization. The data presented in this paper and the one preceding (Wells '15a) as well as that by Shelford and Allee ('13) and Shelford and Powers ('15) show that fishes will react to environmental factors in a way that will tend to remove them from detrimental conditions, long before the adjustment becomes a matter of life and death. Thus we find the salmon leaving the fresh water for the ocean, when, as will be pointed out later, it has been shown (Day '87) that remaining in the fresh water for the entire life cycle would not

result fatally either to the individual or to the species. The mechanism, therefore, which is working to preserve the life of the organism is so delicate that it produces beneficial reactions upon the part of the animal far in advance of life and death complications. The working of this mechanism is undoubtedly closely correlated with quantitative and perhaps qualitative changes in metabolism. These changes in metabolism will have a direct relation to the amount of  $\text{CO}_2$  given off by the organism.

It has been shown that a slight increase in the carbon dioxide content of an animal's blood results in a marked increase in the general irritability, and this increase in irritability would alone result in an increase in the range and vigor of the movements made by the organism. Thus no factor other than increased metabolism need be hypothesized to account for the stimulus which starts the breeding migration of so many animals. The directive factors which result in the animal's coming into special conditions for the breeding activities are another matter. These can be none other than the factors, physical and chemical, which are present in the environment. In the general metabolism of fishes, the stage of development of the sex organs plays an important rôle, and it is very probable that the state of metabolism in these organs furnishes the initial stimulus which causes the animals to start upon the breeding migrations at a given period of the life cycle. Treadwell ('15) points out that the eggs of the Atlantic palola give off an increasing amount of  $\text{CO}_2$  as the swarming season approaches, and concludes that this indicates that there is probably an internal stimulus which is important in producing the swarm. There can be little doubt but that such internal stimulus is acting; the important fact, however, is that it has been shown that such internal changes in the physiological state of the animal may result in very marked changes in the animal's reactions to environmental factors. Allee ('12) has shown that, in isopods, a high rate of metabolism is correlated with a high percent of positive responses to current and that a lowering of the metabolic rate in the animals will diminish and even reverse the rheotactic reaction.

If we consider the different reactions of the salmon to current, acidity, and density, at different stages in the life cycle, beginning with the hatching of the egg we may proceed as follows. It is a well established fact (Loeb '13) that in the fertilized egg and newly hatched fry, the rate of oxidation is high, and it seems to be clear (Wells '13) that from this time on, up to sexual maturity the rate runs down. That is, the rate varies inversely with the age of the fish. Salmon eggs hatched in fresh water must develop into fry which are able to live in slightly acid water, of relatively low density, and the fishes must also be positive to current or they will be swept from the stream. This we find is true and thus ability to live in fresh water is correlated at this time with a high metabolic rate. As time goes by, however, the rate of reaction becomes gradually lower until we find the fishes either becoming actively negative, or at least indifferent to current, and they are swept or swim into the ocean. They now live for some time in the alkaline water of the ocean, and are able to withstand its much higher density. The equilibrium between the environment and the organism is again disturbed after a time, however, and we find the fishes once more selecting the fresh water at another period of high metabolic rate, i.e., with the maturing of the sex glands. From this it would seem entirely possible that fishes which are normally fresh water forms might be temporarily transformed into salt water forms by regulating, that is lowering, the rate of metabolism.

With regard to the selection of the water of greater or lesser density, the data presented in this paper offer an interesting possibility. It has been shown that fresh water fishes whose metabolic rate has been lowered by starvation, will select a notably higher concentration of  $\text{CaCl}_2$  in the gradient than will normal fishes. Also older fishes select a higher concentration than do younger ones. Thus a lowering of the metabolism causes the fishes to choose a medium with higher specific gravity than that normally chosen. It will be remembered furthermore that a stay of a little less than a week in 0.01N  $\text{CaCl}_2$  solution caused a fish that was normally negative to this concentration in a gradient, to become positive. Upon being returned to the tap

water the reaction was again reversed and the fish became negative once more.

Acclimatization of fishes to salts must certainly be concerned with internal adjustments, for Sumner ('07) has shown that the specific gravity of fishes' blood is altered when they are changed from fresh to sea-water, and vice versa. An alteration in the density of the blood seems then to result in a reversal in the reactions of the organism to density in the environment. Green ('04) has shown that changes in the specific gravity of the blood of the salmon occur at the time the fishes are entering the fresh water; the blood gradually acquires a density that averages 17.6 per cent less than that of salmon in sea-water (l.c., p. 454). Jones ('87) has proved that age, exercise, sexual maturity, pregnancy, food, etc., have a measurable influence upon the density of the blood of man, and Sumner ('07) states that there are seasonable differences displayed by fishes, in the osmotic phenomena through their gills. It may be that the specific gravity of the blood of anadromous fishes at different stages in the life cycle, can be used as an index to the physiological changes that are going on in the organism. Also the effect upon the organism of a higher  $\text{CO}_2$  production within the tissues must vary with the density of the blood and would probably be more marked when the blood is less dense.

An investigation of the changes in the density of the blood of the salmon could perhaps best be begun with the fry in the fresh water streams. As the fishes remain for 2 years or even 3 in the fresh water before leaving for the ocean, a thorough study of the relative densities of the blood and the fresh water could be made in this period. That the instinct which causes these fishes finally to reject the fresh water for that of the sea, is backed by some very strong stimulus is indicated by data given by Day ('87). Day speaks of an experiment which was carried on by Maitland in 1880. Eggs of salmon were hatched in fresh water, and the young salmon were placed in ponds shut off from the sea. These fishes ate well and grew vigorously until they were about  $2\frac{1}{2}$  years old. At this stage in the life history, the individuals are known as 'smolts' and it is at the smolt stage that they

leave the fresh water. In October, 1883, one of the fishes jumped out of the pond onto the bank. By the end of November, several had jumped out onto the bank and died there (they usually jumped during the night or early morning). In the following May, 16 of the fishes were found dead on the bank. Then the following October (1884) they commenced constantly jumping out of the pond and meeting with fatal injuries. It was observed that the fishes did not feed at this latter date; this failure to take food is characteristic of salmon entering fresh water to breed.

Examination of the fishes which had jumped out of the pond showed that all were approaching maturity and in the later cases, the eggs and sperm were ripe. An attempt was made to fertilize the eggs with the sperm, with good success. Day states that this second generation was normal and vigorous up to 20 months and concluded that it was definitely proved that a sojourn in salt water is not necessarily for the development of the sexual products. If this is true, the migration of the salmon into the salt water, and back again, is all the more curious. There would be advantages and disadvantages to such behavior but the above data prove that the fishes are reacting to the environment in a way that is not immediately essential though the stimulus seems to be a very strong one. A study of the behavior of these fishes in salt, acid and alkali gradients at different stages in their life history, would undoubtedly prove very suggestive and such a study correlated with physiological investigations of the fishes at similar stages will without doubt solve the question of the movements of anadromous fishes.

#### VI. GENERAL CONCLUSIONS

1. Fresh water fishes recognize and react to the presence of salts in solution. The reaction is one which tends to bring them into their optimum salt concentration.
2. Fresh water fishes (and probably marine fishes also, Sheldford and Powers '15) are not as sensitive to salt ions as they are

to hydrogen and hydroxyl ions. The reactions to either the ions of salts or acids are complicated by the presence of the ions of the other.

3. Fresh water fishes react to combinations of antagonistic salts or to an antagonistic salt and acid, in a way that tends to bring them into a region of optimum stimulation. The phenomena of antagonism are thus indicated by the behavior as well as the resistance of organisms.

4. Starvation causes certain fishes (e.g., *Ambloplites rupestris*, rock bass) to select higher concentrations of salt than those normally selected. Other fishes (*Ameiurus melas*, bull-head) when starved, select lower concentrations than normally. Over-feeding causes bull-heads to select higher concentrations, than those normally chosen.

5. Rock bass and bull-heads which are normally negative to  $\text{CaCl}_2$  0.01N solution, become positive after being kept in this concentration for about a week. They become negative again when returned to tap water for 24 hours.

6. The migrations of anadromous fishes are probably correlated with rhythmic changes in metabolism. These alterations in metabolic activity are largely the result of internal changes such as occur with the ripening of the sexual products.

I am indebted to Prof. V. E. Shelford for proposing this problem and for many suggestions during the work. I am also under obligation to Mr. Karl A. Clark of the Chemistry Department for helpful criticisms and for the loan of apparatus.

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# THE PREDETERMINATION OF SEX IN PHYLLOXERANS AND APHIDS

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FIVE TEXT FIGURES AND TWO PLATES

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## INTRODUCTION

Two of the critical stages in the life of the phylloxerans of the hickories have already been shown to be intimately connected with changes in the cytological relations of the chromosomes. One of these stages involves the formation of but a single class of spermatozoa, that corresponds to the female-producing class of other insects. This fact explains why the fertilized egg gives rise to females only. The second critical stage involves the elimination of chromosomes from the small eggs that are to become males. This fact explains how the male comes to have fewer chromosomes than the female, and brings him into line with other Hemiptera, in which a similar relation holds.

There is a third stage that might also be looked upon as a critical stage; namely, the stage at which the polar body is given off from the egg of the stem-mother; because in *P. caryaecaulis* after that event all of the offspring from one stem-mother are

known to produce large eggs, and all of the offspring of other stem-mothers to produce small eggs. It follows, for this species, either that there must be two kinds of stem-mothers, or else that some external factor must determine that one kind of stem-mother produces daughters all of which contain large eggs, and another kind of stem-mother produces daughters all of which contain small eggs. If such effects are produced by the environment they must be wrought before the mother is mature, because all the daughters of a single female are alike in regard to the size of the eggs that they carry. On the other hand in *P. fallax* some of the offspring of one stem-mother contain large eggs while other offspring of the same mother contain small eggs. In this species there must be only one kind of stem-mother, and either external conditions, or some difference that arises when the polar body of the stem-mother's egg is extruded, must determine whether a given egg becomes a large egg carrier or a small egg carrier.

In either case it became necessary to find out what occurs when the polar bodies of the egg laid by the stem-mother are given off before any further advance in the analysis was possible.

During the last two years I have studied these stages, and I am now in position to give the complete history of the chromosomal cycle. And I can now also give certain additional facts connected with the chromosomes at the time of extrusion of the polar body of the male egg of *P. fallax*. This new evidence makes possible the interpretation of the entire life cycle of the phylloxerans: an interpretation that has a wider interest and application than relates to the life cycle of this group alone.

Before taking up the history of the chromosomes, I may recall the salient features in the life cycle of the two species that are to be considered.

The life cycle of *P. caryaecauleis* is shown in figure 92 in my book on "Heredity and Sex." The stem-mother that hatches in the early spring produces a gall on the leaf of the hickory. As soon as she is mature she begins to deposit her eggs within the gall. From these eggs winged daughters hatch; all those in one gall contain large eggs, all those in another gall contain small eggs. The winged forms leave the galls and deposit their eggs

on the under surface of the leaves of the hickory where they hatch within a few days. From the large eggs emerge sexual females, each of which carries a single egg. From the small eggs, males emerge that are sexually mature at birth. Copulation ensues; the sexual eggs are laid on the stem of the tree. From these fertilized eggs the stem-mother hatches in the following spring.

The life cycle of *P. fallax* is as follows: As soon as the stem-mother is mature she begins to deposit her eggs within the gall. These eggs give rise in this species to wingless daughters (a few winged daughters are also sometimes produced). Whether the daughters in a particular gall are of two kinds, i.e., some containing only large eggs and others only small eggs, is not known, but both kinds of eggs are found in each gall. The large eggs, laid within the gall, give rise to sexual females. The small eggs, also laid within the gall, produce males. The sexual forms that hatch from the eggs crawl out of the gall (whether before or after mating is not known), and the single sexual egg that each female carries is deposited on the tree.

#### THE STEM-MOTHER'S EGGS

As soon as the galls on the young leaves begin to enlarge in the early spring the stem-mother, one in each gall, begins to lay her eggs. If the eggs and embryos contained in the gall are collected and preserved, there is a chance that the last laid egg may be forming its polar body and there is the further possibility that one or more of these may be caught in the anaphase. A very large number of eggs had to be cut into sections before the desired stages were found. One wonders in fact that any eggs are actually obtained in this phase of division. In all I have records of six anaphases that give the desired information.

The initial question is whether there exists a 'lagging' chromosome at this time that might indicate the elimination from some of the eggs of a whole chromosome. A more certain determination would be the count of the chromosomes in the two anaphase plates; but only an extraordinarily favorable case would allow of this being done. Moreover, several such counts

would have to be made in order that the results be significant, for only half of the eggs at most might be expected to show such a chromosome reduction. I think, however, that the presence or absence of the lagging chromosome would make the conclusion reasonably certain. I wish to express here my indebtedness to Miss Edith M. Wallace who has searched through the material and picked out the critical stages. The drawings are also due to her skill.

There are shown in plate 1, figures *a* to *f*, six anaphase stages of the polar spindle of the egg of *P. fallax* and in plate 1, figure *m*, one anaphase stage of the egg of *P. caryaecaulis*. In none of these stages is there any evidence of a lagging chromosome, and since these stages range from a very early anaphase (*a*) to the final stages when the daughter nuclei are reconstructing, there is a strong presumption for the view that no such lagging chromosome occurs. This conclusion tallies, moreover, with the estimated chromosome number. For instance, it was known that in *P. fallax* there are 12 chromosomes in the equatorial plate of the stem-mother's egg, and this same number is characteristic of the somatic cells of the embryo that arises from the egg. If no mistake on the latter counts have been made (the somatic chromosomes are elongated, and, therefore, more difficult to count) there could have been no loss of chromosomes in the polar body. In the other species, however, where only six of the eight chromosomes are apparent in most stages, it might be imagined that loss of one of the attached chromosomes in the polar body, while not affecting the visible count, might so alter the internal relations as to furnish a new point of departure. It would not be profitable here to take up at length the possibilities involved in such a supposition. I have examined them with some care, and have not found that they would furnish any satisfactory solution. On the other hand, the evidence for *P. fallax* is so clear, and the similarity in the two types in all essential points is so evident, that I think we may accept this evidence from *P. fallax* as strongly in favor of the view that all of the chromosomes divide when the single polar body is given off from the egg of the stem-mother.

## THE POLAR SPINDLE OF THE MALE EGG OF PHYLLOXERA FALLAX

In my earlier work I did not obtain any anaphase stages of the polar spindle of the male-producing egg of this species; although a number of equatorial plates were obtained and figured. For certain reasons, that need not now be given, it became evident that this stage in this species should give an answer to certain questions and a long search for anaphase figures was begun. In *P. fallax* the eggs are laid one after the other by each female. Hence not more than a few eggs in the desired stage could possibly occur in a single gall, which renders the chance of finding such a stage very small indeed. Nevertheless, one excellent anaphase was found by Miss Wallace, and is drawn here in plate 1, figure *g*. As shown in the figure, there are two lagging bodies in the middle of the spindle that are conspicuous by their large size. At the inner pole there appear to be ten chromosomes, at the outer pole eight or nine chromosomes.

The equatorial plate from which this figure developed must have contained twelve chromosomes, since this number of chromosomes has always been found present in such a plate. Of these, eight were autosomes and have divided, so that eight daughter chromosomes go out into the polar body and eight remain in the egg. The four sex chromosomes, that are presumably paired at this time remain to be accounted for. If the two bodies seen in the middle of the spindle are two whole X chromosomes, then there can be but eight in the outer plate (which becomes the polar nucleus). There will be ten chromosomes at the inner pole of the polar spindle. But if the two lagging chromosomes represent a single X chromosome, precociously split in two, there will be nine chromosomes in the outer plate (which becomes the nucleus of the polar body). There will be as before ten chromosomes at the inner pole of the spindle. Unfortunately it is not possible to determine, with certainty, whether there are eight or nine chromosomes in the outer plate. I am inclined, nevertheless, to adopt the former interpretation as the more probable, because of the evidence from *P. caryaecaulis* that bears on the same point. It will be observed that *the end*

result is the same whether the lagging bodies represent two X chromosomes, or one X chromosome precociously split, for on either view eight (half) autosomes and two whole chromosomes (X) pass out of the egg, leaving eight half autosomes and two whole (X) chromosomes within the egg.

The polar bodies of three other male-producing eggs are also shown in plate 1, figures *h*, *i*, *j*. These show one or two dark staining granules outside of the nucleus of the polar body, which I interpret as the remains of the lagging chromosome. In this respect they behave in the same way as do the lagging chromosomes in *P. caryaecaulis* which also fail to enter the nucleus of the polar body. A fourth drawing, figure *k*, represents a late stage in polar body formation in which twelve chromosomes are distinctly counted in the inner nucleus of the polar spindle. The outer nucleus is only partly present in this section, and the rest of it could not be found in the neighboring sections. This egg must have been a female-producing egg in which there is no lagging chromosome, and in which there are twelve chromosomes at the inner pole.

Bringing these facts into relation to those already made out, we can construct the chromosome cycle of *P. fallax*, and with this as a clue make clear the similar cycle of *P. caryaecaulis*.

#### THE CHROMOSOME CYCLE OF PHYLLOXERA FALLAX

The main phases in the cycle are as follows:

1. There is a single kind of stem-mother in this species whose eggs contain twelve chromosomes that divide when the single polar body is given off, so that twelve chromosomes pass out and twelve remain in the egg (plate 1, *a* to *f*).
2. These eggs develop into wingless females that produce large and small eggs, but whether the same female produces large and small eggs could not be determined. Both kinds of eggs are found within the same gall, and therefore come from daughters of one original stem-mother.
3. The large egg—the so-called female-producing egg—gives off one polar body, all the chromosomes dividing at this time (plate 1, *k*). This egg develops into the sexual female. The

small egg also gives off only one polar body (plate 1, *g, h, i, j*). At this time the four sex chromosomes conjugate, so that two whole chromosomes pass out into the polar body, as lagging chromosomes, and two remain in the egg while all the other chromosomes divide. The male that develops from this egg has ten chromosomes.

4. The sexual female produces but one egg, the reduction in the number of chromosomes taking place at this time (plate 1, *l*). Presumably two polar bodies are given off, leaving six chromosomes in the egg.

5. In this species the male has ten chromosomes, two of which are X chromosomes. During the first spermatocyte division these two chromosomes pass into the functional cell, so that all the functional sperm come to have six chromosomes.

6. When these sperm fertilize the sexual egg the total number of chromosomes is again brought back to twelve.

#### THE CHROMOSOME CYCLE OF PHYLLOXERA CARYAECAULIS

The main relations of the chromosomes in this cycle are illustrated in diagram 1. There are four ordinary chromosomes or autosomes (colored black) and four sex chromosomes (represented by open circles). Two of the sex chromosomes are as large as the autosomes and two are much smaller. The latter are in most stages loosely united to the larger sex chromosomes, one to each. One of the small chromosomes is *marked* by two cross lines in order to distinguish it from the other one. I have marked it in this way because, as will be shown, an important fact in the life cycle of this species can be accounted for, if one of the two small sex chromosomes is different from its mate. To the left in the diagram the line culminates in the sexual egg. As seen to the right, the line derived from another stem-mother (the one that contains the marked X) leads to two kinds of males, each of which produces its particular class of spermatozoa, one kind containing the open x and the other kind the marked x.

If we first follow down the female line to the left, we see that the egg laid by the stem-mother contains eight chromosomes.

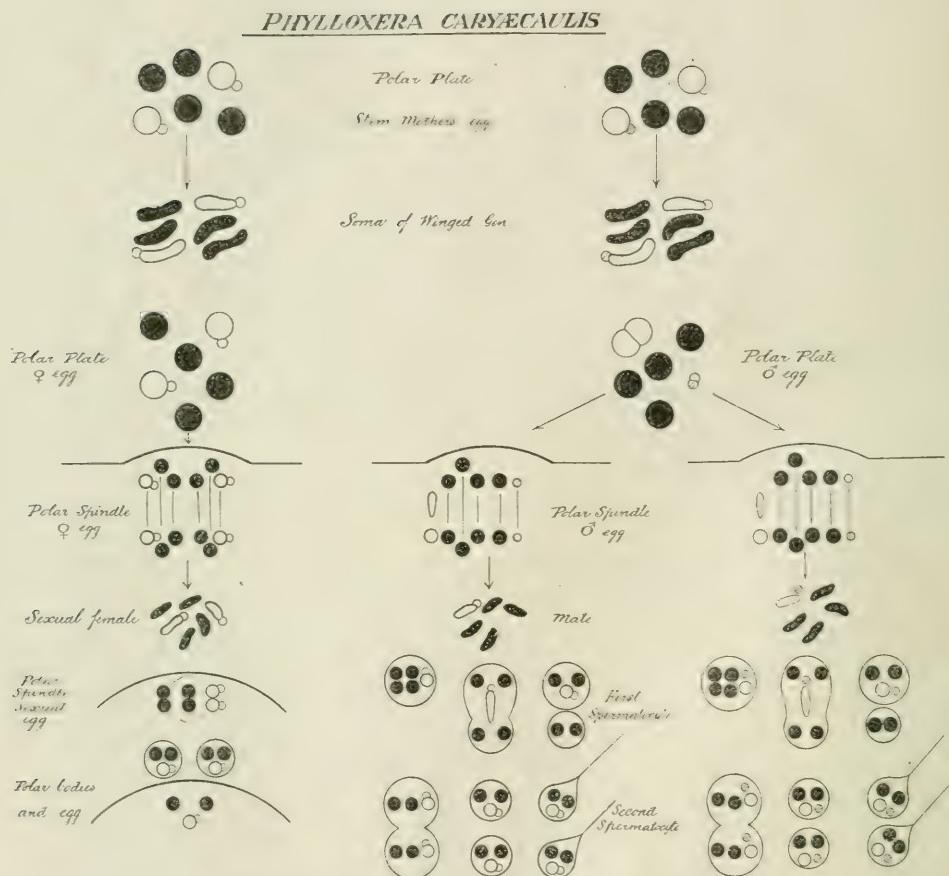


Diagram 1 Illustrates the chromosomal cycle of *Phylloxera caryaecaulis*; to the left is shown the line that culminates in the sexual female; to the right is shown the line that culminates in the males; this line splits into two subdivisions after the extrusion of the polar body in the male-producing egg.

When the polar body is thrown off all of the chromosomes divide. The migrant that develops from this egg comes to produce large eggs with the full complement of chromosomes in them. All of the chromosomes divide when the polar body is produced by this egg (plate 1, fig. *n*). The egg itself then develops into the sexual female that comes to produce a single egg. When this egg is ripe the chromosomes have united in pairs, of which there are three. In reality one of these pairs must be thought of as made up of the two large sex chromosomes and the two small chromosomes attached to them.

If we next turn to the line represented on the right of the diagram we see that the stem-mother contains the same group of chromosomes as in the other case (one of the small sex chromosomes is marked). All of the chromosomes divide when the polar body of the egg of the stem-mother is given off. The egg gives rise to the migrant that comes to produce the small eggs. When the polar body is about to be produced in these small eggs (or before that event) a shifting of the sex chromosomes takes place, so that the two large X's come together as do the two small ones, the latter leaving their former loose attachment to the large chromosomes in order to combine. When the polar body is given off the four autosomes divide, while the conjugated pairs of sex chromosomes separate; a member of the larger pair lags on the spindle and is precociously split lengthwise, while the small pair separate and the outer member does not lag on the spindle (plate 1, fig. *o*). It will be seen that two kinds of eggs should result according to whether the marked chromosome passes out into the polar body, or remains in the egg. If it passes out, the male that results will give rise, as shown in the figure, to female producing spermatozoa that contain the open x. If we suppose the sexual egg is fertilized by this kind of spermatozoan, the stem-mother that results will give rise to the female line (to the left). If we suppose that the other kind of sperm—the one with the marked x—fertilizes a sexual egg, a stem-mother will be produced that leads to the male line (to the right). The assumption that there are two kinds of males is not entirely hypothetical; for, in my former paper I pointed out

that two kinds of males are found that differ from each other in the relation of the small  $x$  to the large  $X$  chromosome—in one kind of male the two remain in contact, in the other the two keep apart. And I pointed out that all of the spermatocyte cells of a given individual show one or all show the other relation. This is in accord with the assumption of two kinds of males that arise when the polar body is eliminated.

In some of the preparations showing anaphase figures in this species, one of which is redrawn here in plate 1, figure 9, the two lagging chromosomes appear unequal in size. In other figures, however, (figs. 5, 6, 21, of my 1912 paper) the two lagging chromosomes appear to be equal or subequal. For this reason I stated ('12) that the two equal chromosomes represent one large sex chromosome divided into halves. On this view the conjugating pair of small  $x$ 's have been separated and moved to their respective poles while only the large  $X$  lags as it passes to the outer pole.

It will be noticed that superficially the number of chromosomes in *P. fallax* is double that in *P. caryaecaulis*. This suggests that the group in *P. fallax* represents the double number of chromosomes of *caryaecaulis* (tetraploid), or that *caryaecaulis* is *P. fallax* halved. But if the full number of chromosomes in *P. caryaecaulis* is looked upon as eight, this relation does not hold, unless one supposes that there are four small chromosomes present also in *P. fallax* (giving sixteen in all) that are attached permanently to other chromosomes. From this point of view the chromosomes of the one species would be double the number of the other and *P. fallax* would become XX and XY, while *P. caryaecaulis* would become X and Y. In other words the small chromosomes would no longer be reckoned as factors in the sex scheme. I have preferred, however, the interpretation given in the diagram because the two small chromosomes conjugate in the male egg when the large sex chromosomes conjugate; and, again, because one of them lags in the first spermatocyte division along with the lagging X. But the latter relations may be only a consequence of the first, and be due to the absence of a mate at this time. Still, since it goes into the female-producing sperm, and

not indifferently to either pole, this would seem the more natural designation. Without wishing, therefore, to lay too much stress on the nomenclature, it seems to me that the one I have followed represents more naturally the facts as described in the text.

#### SEX RATIOS IN PHYLLOXERA FALLAX

Since the galls in this species do not open to release the sexual forms until a considerable number of them have hatched, and since all of the inhabitants (except for rare cases) are the descendants of a single female it is possible to get a fairly good sample of the output of each stem-mother. In order to anticipate a possible objection, viz., that two or more stem-mothers might be included within the same gall, I opened a number of very young galls and found, with the rarest exceptions, that each gall is the result of the activity of a single female. We are safe in concluding, therefore, that the contents of each gall is the product of a single stem-mother. But whether her daughters, the apterous 'migrants,' are individually small-egg-producers or large-egg-producers can not be determined; because, in this species, the apterous 'migrants' deposit each egg as it matures. Since eggs that are not quite mature look like small eggs they can not be distinguished from them. Only by finding a gall in which a single apterous 'migrant' was present that had laid both kinds of eggs would it be possible to settle this question. I have already reported that occasionally the migrants are winged, and that when this occurs each individual contains eggs that were all of one sort, namely, small eggs in this instance, but this does not settle the other question, however probable it may appear, that each individual of this generation produces only one sort of egg.

In the following table the counts from 26 galls (July 11) are given. Those with the same letter come from the same leaf. The galls were old and ready to open. The old apterous 'migrants' were still present, but empty, as though they had about finished their productive life. There were present as many eggs unhatched as hatched; only the latter appear in the record, or

rather the individuals that hatched from them. In some of the galls the dwarf forms recorded in my former paper were also found.

Except in a few cases the females were in excess of the males. If galls are opened, when the first sexual forms have begun to hatch, males (one, two, or three, etc.) will be found. This means,

TABLE I.

	MALES	SEXUAL FEMALES
OP	28	36
OP	20	31
OP	6	14
OP	15	2
OP	30	15
TT	4	11
TT	18	32
TT	29	39
TT	5	6
TT	21	28
TT	15	26
SS	24	33
SS	21	23
SS	2	7
SS	9	18
SS	2	2
SS	14	17
SS	12	28
SS	18	12
	4	27
	10	21
WW	7	51
WW	17	30
WW	30	32
WW	8	21
WW	36	66
	405	628

no doubt, that males hatch first, or possibly that the first eggs produced are males. Since later more females than males are present, it follows that more female-producing than male-producing eggs are laid. The most striking departure from the ordinary ratios is that in the first count in WW where there were 7 males to 51 females.

Galls of the same size on the same leaf must have been subjected to as nearly uniform conditions as possible, but the counts from the same leaf are not strikingly at least more like each other than they are like other counts from other leaves.

It is idle perhaps to speculate as to the factors that predetermine whether a given egg shall be a large or a small one especially as this may depend, as in the other species, not on the conditions that affect the migrant, but on the conditions that determined the nature of the migrant herself. The recent work of Whitney and of Shull shows that very slight differences in the environment turn the scale in sex predetermination. Differences like those described by them might accompany the differences in food conditions of the leaf during the day when starch is being made and during the night when starch is being converted into sugar. If, perchance, such environmental changes affect the stem-mother, the differences as to output shown by the galls, might arise.

#### THE SPERMATOGENESIS OF THE BEARBERRY APHID

Stevens and von Baehr have described very completely the spermatogenesis of several species of aphids. I have also studied at different times a number of species but as they gave nothing new I have not published the results, except for one very brief account (*Jour. Exp. Zoöl.* '09. pp. 298-305). There is one species, however, which in clearness and simplicity exceeds all others that I have seen. I give here therefore an account of some of the critical stages in its spermatogenesis and oogenesis. The data furnish, moreover, an occasion to make certain comparisons between the phylloxerans and the aphids.

The species in question, *Phyllaphis coweni* Cockerell, forms galls on the bearberry. My material was found north of Quissett, Mass., near Woods Hole, where I have collected material for four summers. I have found the galls on the bearberry during June, July and August. Each contains, as a rule, a single stem-mother—rarely two—and her progeny. As the gall gets older the progeny is seen to be made up of larger individuals with wing pads and in addition a series of immature forms, as well as a few males. The large individuals with wing pads contain sexual

eggs. As I have never found winged individuals within the galls the individuals must leave the galls when ready to expand their wings. The origin of the males puzzled me for some time until I discovered that the stem-mother produces them—at first sparingly, but later in larger numbers. The same stem-mother that contains the males also contains young sexual females. She also produces at times both males and individuals that contain parthenogenetic eggs and embryos with six chromosomes.

Throughout July and August young galls can always be found at the growing ends of some of the branches. These galls contain young stem-mothers, and later some of their progeny. These younger stem-mothers may possibly come from the old stem-mothers, or from belated eggs of the previous year, or from sexual eggs of the same year in which they appear. A more detailed examination will be necessary to settle this point.

The sexual eggs begin to pass through their synapsis stages while the young are still present in the stem-mother but even after the young are born and after some of the eggs have left the ovary, eggs at the outlet may show the chromatin contracted at one side. In the male the two reduction divisions may also take place while the young individual is still within the stem-mother, but other individuals do not contain these stages until after the young males have been born.

Entire individuals were preserved in Carnoy solution, the abdomen cut into sections, and the sections stained in iron haematoxylin.

The early spermatogonial cell contains five chromosomes (diagram 5). At a later stage I found what seems to be a contraction figure, plate 2, figures 1 and 2, when the chromatin is shrunken and lies at one side, but as there is nothing specific about this condition it is with some hesitation that I identify it as the synizesis stage.

The early prophases, plate 2, figures 3, 4 and 5, are interesting. The three chromosomes are easily distinguished, even before they shorten into rods.

Miss Stevens has described a stage that she calls the synapsis stage; but from her figures it seems not improbable that she has

seen only the early prophases of the first division or possibly the stage between the first and second spermatocytes.

The side view of the first spermatocyte division is shown in plate 2, figure 6. An equatorial plate is shown in plate 2, figure 5, which gives the relative sizes of the three chromosomes.

Stages in the later first division, some of them duplicates of each other, are shown in figures 7 to 11. The two autosomes divide; the X chromosome is drawn out, and is usually dumb-bell-shaped. It generally shows a well marked longitudinal split. The split is so distinct that the halves appear often like two parallel lagging chromosomes. Only in the latest stages of the division is it apparent that the whole X chromosome passes into the larger of the two cells. As in other aphids, the X chromosomes are, during this division, often constricted near the middle, which in some species is sometimes carried so far that the two enlarged ends are connected by a mere strand. It is this appearance that has led Miss Stevens in her earlier work to infer that the X chromosomes were really divided at this time. In the aphid of the bearberry the constriction appears at first at the middle of the chromosome. It seems later to pass more and more towards one end, until ultimately, as shown in figures 11 and 13, a small piece only is left at one end, which in most cases is later drawn into the thread; although once or twice I have found cases, as in figure 12, in which this terminal piece appears to have broken away from the strand connecting it with the rest.

The difference in size of the two cells varies greatly in the earlier phases, as the figures show. But ultimately nearly all of the protoplasm passes into one cell, the one that contains the X chromosome. The small cell is left with two chromosomes and a small amount of cytoplasm. It never divides again, and later degenerates. Stevens was inclined to think that the small cell may sometimes show a division figure, which subsequently fades away, but I have never seen a case of this kind. The two autosomes in the functional cell begin to lose their condensed condition and spread out into loose masses, as shown in figures 16 and 17. The X chromosome is later in passing into this con-

dition, but does so before condensation sets in again, preparatory to the next division. In one case, figure 15, the X chromosome failed to draw out of the smaller cell, and one end only lies within the nucleus of the larger cell. This end has begun to open out as the other chromosomes have already done. The condition of the other cells in the cyst makes this interpretation probable.

During the resting stage, the X chromosome becomes condensed, as shown in plate 2, figures 14, 15, 16, 17. The chromosomes in the larger cells next begin to condense, preparatory to the second division. The X chromosome is distinctly larger than the other two; all three divide equally to form the spermatids (plate 2, figs. 18, 19, 20).

The double nature of the X chromosome in the first spermatoocyte division is so apparent that it invites speculation concerning the nature of the division. Presumably the second division occurs in the plane of the split, but it is impossible to follow this chromosome through its resting stage. Similar cases for the single X chromosome are known, and I can but follow the usual interpretation, viz. that we are dealing with a precocious division. The tetrad formation that occurs in such forms as *Ascaris* is interpreted as a double division, one of which is precocious. The rapidity with which the two reduction divisions take place, often without an intervening resting stage, indicates that each of the chromosomes, even though mated in pairs, has undergone the preparatory stages of division. Hence two divisions are necessary to separate the four elements. But if this were the whole of the matter it is not apparent why, in a case like this one, the halves of the X chromosome do not separate from each other at the first division. It is perhaps little more than an evasion of the difficulty to suggest that the divisions in the X chromosome are not sufficiently advanced, when overtaken by the first division.

Janssens has proposed a view of the necessity of the two reduction divisions, based on his observations of the chiasma type. His studies of *Batracocephalus* have shown that two of the four threads of the tetrad sometimes break and reunite so that two

new threads are made up of parts of each of the two original threads. Under such circumstances a single maturation division would often produce a dyad in which the two threads are genetically unlike. Janssens assumes that this is repugnant to the scheme of reduction, whose purpose is to give rise to a gamete with a single set of units (gens). This solution of the problem rests on the supposed necessity of pure gametes, and this in turn could be more directly accomplished, it would seem, if the conjugating chromosomes separated without interchanging parts, as they do, in fact, in the male of *Drosophila*. Granting, however, that 'crossing over' does occur as a necessity of the physical conditions prevailing at this time, Janssens' hypothesis might appear to furnish a solution, if at the same time the necessity for pure gametes could be shown essential to development. Obviously, however, the act of conjugation is a capital arrangement to give the zygote a heterozygous make-up, and why a quadruple set of gens instead of a double one would be a disadvantage is not self-evident.

#### A TETRAPLOID CYST IN THE BEARBERRY APHID

In a male of the bearberry aphid one cyst was found in which all the cells have the double number of chromosomes including the sex chromosomes. The cyst is in the first spermatocyte stage. Since the other cells in the same testis and in the testis of the other side are normal the tetraploid condition must have arisen from a spermatogonial cell whose chromosomes but not the cytoplasm divided. The other possibility, namely, that the four autosomes failed to conjugate, would not account for the presence of two X chromosomes, nor explain the doubling in all the cells of the cyst, because conjugation occurs long after the cells of a cyst have become separated. The former interpretation is therefore to be preferred. The cells in this cyst, of which a few are shown in plate 2, figures 21-30, are completing, or else have completed, the first spermatocyte division. Taking the figures in order, we see in figure 21 four autosomes at each pole—only three show in the larger cell—and two X's extending from

one nascent nucleus to the other. Practically the same relations are shown in figure 22, where however, the two X's appear to be passing into the larger cell. In figure 23 the two X's stand end to end as is also the case in figures 24 and 25. It is not possible to determine into which cell they might have passed—presumably, however, one to each cell. In figure 26 this result seems to have been attained, since each cell contained four autosomes, and one X. In figures 27, 28, 29 and 30, the division having been accomplished, only the larger cell is shown. In each case there are two X chromosomes and four autosomes in the cell.

There are some questions here of theoretical interest. The autosomes appear to have acted as pairs, if one may judge by the equal distribution to the daughter cells, which seems to have taken place in many cases, although it can not be established for all cases. As there are four autosomes of each kind their copulation in pairs does not seem unexpected. The behavior of the X chromosomes is unique. In some cases they have passed to opposite poles and in this sense have acted as a pair, but in most cases they have passed into the larger cell. The first spermatocyte division in phylloxerans and aphids is of such a kind, that the X chromosome passes into a particular cell, i.e., it does not pass indifferently to either pole. But as a matter of fact we do not know whether the larger cell into which it passes is predetermined (by some polar relations in the cell) and the X chromosome follows this preexisting condition, or whether that cell becomes the larger one, which happens at the time of division to contain more of the X chromosome (owing, let us say, to its accidental excentric position). If one may judge from the appearance of the early stages of the first spermatocyte division, the former alternative may seem more plausible. If this be the correct interpretation then the more usual case of the two X's going to the same pole in the abnormal cyst would be due to their relation to a particular pole of the dividing cell. The exceptional passage into the other cell would be due to one of them getting caught by the constriction, so that it was necessarily detained in the smaller cell. But the more nearly equal sizes of the

cells in the cases here figured, when an X passes to each pole, may seem rather to favor the other view, namely, that the size of the cell is determined by the chance direction taken by the X chromosome.

If cells like these with duplex number of chromosomes should produce functional spermatozoa, and one such spermatozoon should fertilize a normal egg, the number of somatic nuclei would become nine instead of six and the resulting female would have three X chromosomes instead of two. In subsequent generations the number of X's might be further increased, if, in fact, viable forms could be produced in this way. It does not seem probable, however, that a condition like that described above for the phylloxerans, in which four X's are present, could have arisen through irregularities of this kind, because the chance that such a rare phenomenon could supplant the normal type seems too small to make such a view possible. Still, the possibility of a tetraploid organism, arising through failure of a spermatogonial or oogonial cytoplasmic division must be conceded, especially in the light of the sudden appearance of tetraploidy in *Primula* and *Oenothera*. If it be assumed that some advantages, such as an increase in size, give the new type an advantage, then it might in time obtain an independent footing.

A most striking and interesting relation is shown in this tetraploid cyst, namely, the chromosomes are only half as large as are those at the corresponding stage of the normal spermatocyte stage, as seen in other cysts of the same testis. This relation might be interpreted to mean either that the original mother-cell of the cyst, having divided (incompletely) one time more than the other mother-cells of the other cysts, never made good the size loss of the chromosomes. If the growth of the chromosomes be directly related to the size of the cell that contains them, owing to the amount of substance available in such a cell, the smaller size of the chromosomes in the tetraploid cyst might find a reasonable explanation. Such a conclusion would indicate that the stage reached by a cell at a particular phase is determined by the cytoplasm, rather than by the size of the chromosomes.

THE OMISSION OF SYNAPSIS IN THE PARTHENOGENETIC EGGS  
OF PHYLLOXERANS AND APHIDS

As is well known, the full or diploid number of chromosomes is present in most eggs that develop by means of parthenogenesis. Whether the presence of the full number of chromosomes has in itself anything to do with the phenomenon of parthenogenesis may well be disputed, because in some forms, as in the male bee, the eggs develop without being fertilized with half the number of chromosomes and in artificial parthenogenesis the half number of chromosomes occurs in some forms, at least. In the phylloxerans and aphids there is a loss of one or two chromosomes from the male-producing egg that develops by parthenogenesis.

There is a further question that is important from a descriptive cytological point of view, namely, whether the parthenogenetic eggs omit the synapsis stage and retain in consequence the full number of chromosomes, or whether they pass through such a stage and the chromosomes subsequently separate. In phylloxerans and aphids the case is quite clear<sup>1</sup> and I wish to emphasize the ease and certainty with which the problem can be studied in them.

In the bearberry aphid, the ovary that is producing sexual eggs (diagram 3) can with certainty be distinguished from the ovary that is going to produce parthenogenetic eggs (diagram 2). In the latter there is no contraction phase of the chromosomes. A prophase of an oogonial division of a parthenogenetic egg is shown in diagram 2, *a*. Six chromosomes are distinctly seen and the same number is found in the equatorial plate stage shown in diagram 2, *b*. At the beginning of the growth period, when the chromosomes begin to take the stain again, scattered threads or strands can be made out, as shown in *c*, which by further contraction, *d*, give rise finally to the six rod-like chromosomes. In later stages, when the egg is about ready to leave the ovary and after that time while it is still acquiring yolk, the six chromosomes can be distinctly seen and easily counted, as shown by most of the eggs in diagram 2, *e* and *f*. In the ovary of the sexual individual the chromatin begins to condense into threads at the

<sup>1</sup> Morgan, T. H. Proc. Soc. Exp. Biol. and Med., vol. 7, 1910.

beginning of the growth period, as shown in diagram 3, *a*, *b*, *c*, and the threads later condense at one side of the nucleus, as shown in *d*. No details of the process of union of the chromosomes, that must take place at this time, can with certainty be made out. The figures are as accurately drawn as possible, but

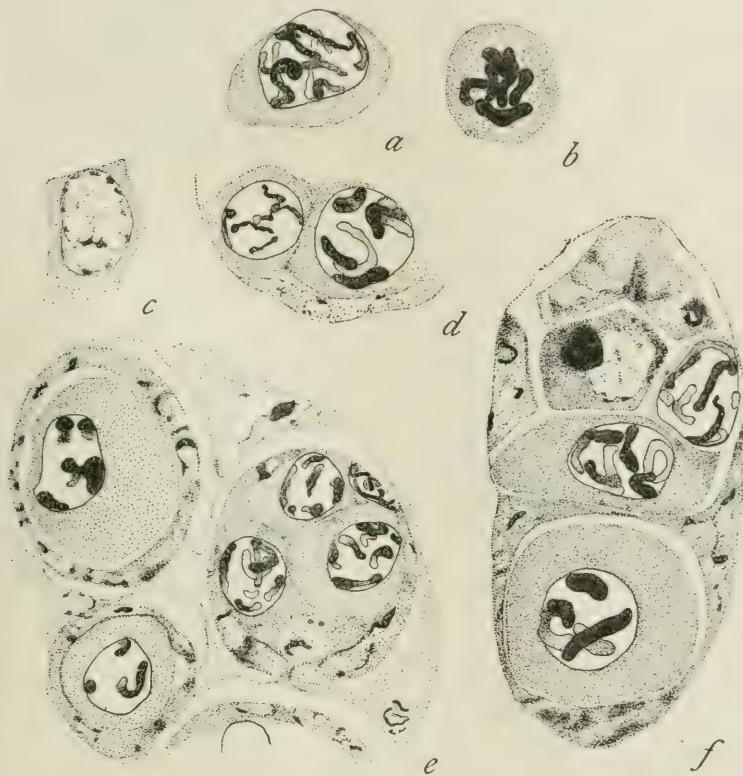


Diagram 2 *a*, prophase of an oogonial division; *b*, metaphase of an oogonial division with six chromosomes; *c*, young parthenogenetic ovum just prior to the appearance of the chromosomal filaments shown in *d*, *e*, *f*, young ova for the most part in the lower end of the ovary or else just out of the ovary, showing six chromosomes whenever all of the chromosomes are included in the section.

beyond the fact that the chromosomes are condensing, the interpretation of the details of the drawings is unsafe. When the chromosomes begin to emerge from the condensed stage, as seen in diagram 3, *e*, *f*, *g*, *h*, three clumps or rods, or sometimes open

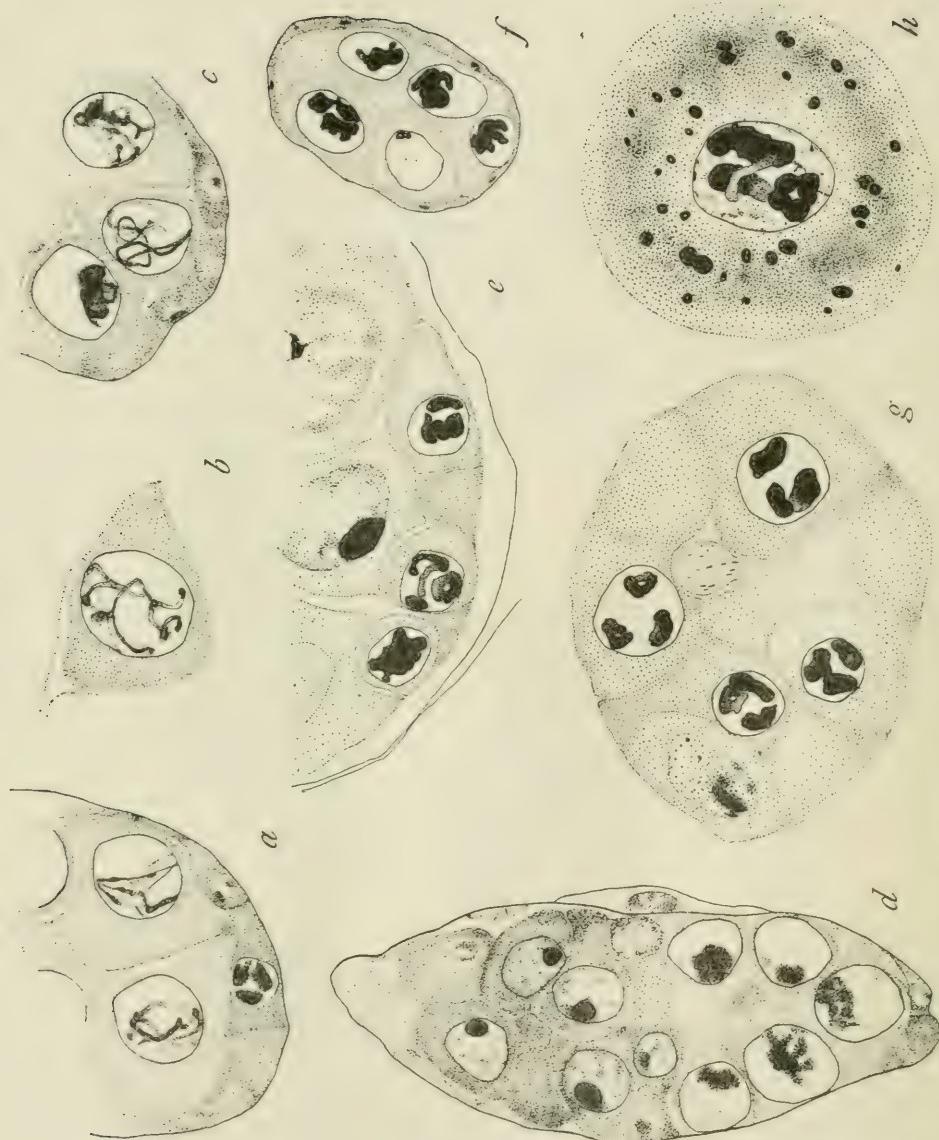


Diagram 3 *a, b, c*, cells in the posterior end of the ovaries of sexual individuals of the bearberry aphid, showing young ova passing into synapsis; *d*, section of whole ovary with nutritive cells in anterior end and eggs in synapsis stage at other end; *e, f, g, h*, young ova in which the three chromosomes are emerging, or have emerged from the synaptic stage; *h*, young ovum before the polar body is formed.

rings, appear and in this condition they are carried onto the polar spindle.

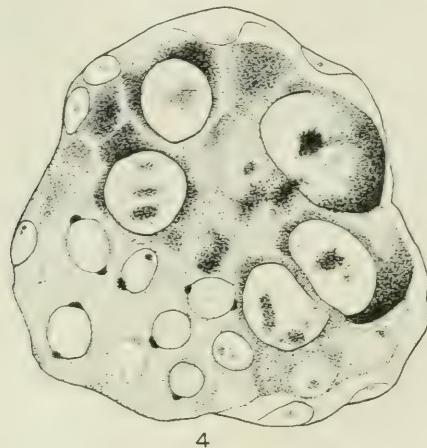
In the bearberry aphid, the stem-mother produces many male embryos as she gets older. Their presence in the mother serves as an index of the condition of her ovary at this time. An examination of her ovarian eggs at this time failed to show in the chromosomes any process suggestive of synapsis, although no doubt the steps preparatory to the elimination of the two sex chromosome must be taking place at this time. As only two chromosomes are involved it is quite likely that even if they went through a contraction phase independently of the rest of the chromosomes (if such were possible) it would be difficult to recognize such a process. The negative evidence has no special value and is mentioned here only to show that the stages were examined for evidence of synapsis.

In the phylloxerans the ovary of the stem-mother continues throughout her life to produce a series of eggs that develop by parthenogenesis. All stages in the development of the eggs can be found in almost any female. There is never in any of them the slightest evidence of a contraction phase, and, since the eggs show exactly the same conditions as do the parthenogenetic eggs of the bearberry aphid, it will not be necessary to repeat here what has already been said. The ovary of a young female that will later reproduce by parthenogenesis is represented in diagram 4.

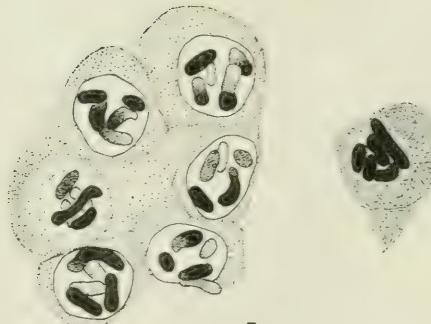
The migrant generation of the phylloxerans also produces eggs that will develop by parthenogenesis. In *P. caryaecaulis* all of the eggs develop at nearly the same time, so that the conditions for the study of parthenogenetic stages are not so favorable as in *P. fallax*, where the wingless 'migrants' produce one egg at a time over a considerable period. In neither species have I seen any evidence of contraction, nor have I seen any other evidence of conjugation of the sex chromosomes, with the exception already noticed where two chromosomes of double size were found in two eggs that would give rise to male embryos later. Here, however, the nucleus was ripe and ready to take part in the formation of the polar spindle. The observation only shows that

the two pairs of conjugated chromosomes have already united before the spindle is formed.

Von Baehr describes for *Aphis saliceti* a synapsis stage in the spermatogenesis in which the chromosomes are contracted at one



4



5

Diagram 4 Section of ovary of parthenogenetic female of second generation, with large nutritive cells at one end and small egg cells at the other end on the ovary.

Diagram 5 Spermatogonial cells in prophase stages to left and one cell in metaphase to right; all cells show five chromosomes.

side of the cell. As no further details that identify this stage were made out, one can not be certain that such figures represent the true conjugation stage. I have studied the spermato-

genesis of several species of aphids in the hope of getting better figures of this stage, but have found none better than those given by von Baehr. In those species where the formation of the spermatozoa is a continuous process in the testis of the adult male it is possible to obtain in the same testis all stages, from the dividing spermatogonial cells to ripe spermatozoa. A contraction figure is a constant occurrence just after the spermatogonial stages. The presumption is that here, as in the bearberry aphid and in *Aphis saliceti*, we are dealing with a true synapsis stage, but as I find a contraction figure often present in older cells also, almost up to the time of the spermatocyte stage, I can not feel that the criterion of the contraction figure alone suffices to identify this stage with certainty, especially when in other forms, as in *Lagus*, a second contraction (not conjugation) stage has been recognized.

#### HISTORICAL RETROSPECT

In Feb. 1908, I described the formation of the functional female-producing spermatozoa of the phylloxerans and also the degeneration of the male-producing spermatozoon. The consequences of the formation of a single female-producing class of sperm were pointed out. At that time I had also studied many preparations of aphids (my own as well as some of Miss Stevens') and reached the conclusion that in them too the same process occurred. I did not publish this conclusion, because I wished to give Miss Stevens the opportunity to correct her earlier statement in regard to the spermatogenesis in the group. This she did in the paper of 1909. W. B. von Baehr had also been studying with Boveri the spermatogenesis and oogenesis of aphids and had come independently to the same conclusion that I had reached. He published his preliminary paper in the *Zoologischer Anzeiger*, October 13, 1908, referring there to his own as similar to my results in the phylloxerans, and the complete paper in 1909.

In Science (Feb. 5, '09) I reported further facts connected with the spermatogenesis and the number of chromosomes in the male- and in the female-producing eggs. In September of the

same year I published a full account of my work. In 1912 I obtained evidence that showed how the male-producing egg sent out a whole undivided chromosome (or two whole chromosomes) into the polar body. The number of chromosomes being diminished, a male develops.

Another paper by Von Baehr appeared in 1912 in *La Cellule*. A detailed account of the spermatogenesis of *Aphis saleceti* is given, but no new facts of any importance were added. Certain details in this paper will be referred to in other connections.

In my 1908 paper I pointed out that the male in the phylloxeran had one less chromosome than the female, in contradiction to Stevens' earlier conclusion that the same number of chromosomes is present in the male and the female. I added "It seems probable that the change takes place in the formation of the single polar body given off by the parthenogenetic egg." Von Baehr also described one less chromosome in the male than in the female, but, in his earlier paper, he like Stevens and myself, had made no observations to show where or how the loss takes place. In von Baehr's full paper in 1912 also, there are no critical stages to show how the elimination is brought about. Stevens found in 1910 two polar 'plates' in the male-producing egg, containing one less chromosome than in the parthenogenetic female. She suggested that two chromosomes had united in anticipation of the reduction division when the polar body forms. Whether this union does really occur in the aphid has not yet been shown, nor has the presence of a lagging chromosome been seen as yet, but from analogy with the phylloxerans there can now be little doubt that a whole chromosome is lost in the polar body of the male-producing egg, and that a preliminary union of the two X chromosomes may take place. In *P. fallax* I have already recorded two cases in which four chromosomes met to produce two pairs ('09, p. 246, figs. u, w), and from the shifting of the chromosomes, or rather from the size relations, I inferred that such a union must also take place in *P. caryaecaulis*.

Stevens' identification of the synapsis stage in her 1905 paper I hold to be erroneous, because what appears to be the true sy-

napsis in other forms does not in any way resemble the process that she describes as such. The four figures that Miss Stevens gave to illustrate the stage in question are quite inadequate to establish this interpretation. Von Baehr also dissents from Miss Stevens' view.

In parthenogenesis the relation between reduction in number of the chromosomes and the occurrence or non-occurrence of the synapsis stage has been studied in a few forms. Woltereck in 1898 examined the oogenesis of a species of ostracod, *Cypris repans*, known to breed by parthenogenesis, and showed that the eggs have a distinct contraction stage ('synapsis'). Prior to this condition the chromosomes thicken and then condense at one side of the nucleus. When they emerge, the full number is present, i.e., there has been no reduction through pairing of the chromosomes. Since this change takes place at the time when the ordinary synapsis would be expected, there is a presumption in favor of the view that the contraction figure corresponds in some regards at least to the synapsis stage.

Schleip in 1909 studied both parthenogenetic and sexual species of ostracods. In both a synapsis stage was found from which in the parthenogenetic species the full number of chromosomes emerge; while in the sexual species the reduced number of chromosomes appear.

Kühne in 1908 described a stage at the beginning of the growth period in the parthenogenetic phyllopod, *Daphnia pulex*, in which the chromosomes contract and concentrate in radial lines around the nucleolus. He compares this condition with the synapsis stage described by Woltereck, but thinks that the evidence here does not suffice to establish the identity of the two. The total number of chromosomes appears later, without any evidence that there has been pairing.

Fries in 1909 found no synapsis stage in the phyllopod, *Artemia salina*, which reproduces by parthenogenesis. The egg and the body cells contain the full number of chromosomes. In *Branchipus*, on the other hand, which reproduces by means of sexual eggs, there is a usual synapsis stage, followed by reduction in the number of the chromosomes.

Tannreuther in 1907 studied the maturation of the parthenogenetic and sexual eggs of the aphid, *Melanoxanthus salicis*. He found the full number of chromosomes in the parthenogenetic egg, and pointed out that no previous reduction stage was present. His study of the earlier stages of this egg does not seem to me to suffice to have precluded the possibility of a synapsis stage being present, especially in the light of his wrong identification of that stage in the sexual egg. Tannreuther missed the reduction stage of the sexual egg, but describes at length a process that he calls "reduction of chromosomes." This, he says, occurs after the egg has left the ovary, but these stages can have nothing to do with the synapsis for the pairing has already taken place before this time. He also overlooked the synapsis stage in the spermatogenesis; for, the pairing of the chromosomes which he describes is a much later stage—probably a stage when the reduced chromosomes are emerging for the first spermatocyte division. The behavior of the lagging chromosome was wrongly described and interpreted. My own observations relating to synapsis in aphids and phylloxerans were published in 1910. Grégoire in 1910 has given in an admirable résumé an account of what has been done in connection with synapsis and reduction in parthenogenetic eggs. In particular, he has drawn attention to the similarities between the accounts of synapsis in parthenogenetic eggs and apogamy in certain plants, where, according to Strasburger, synapsis may occur, but fails to bring about reduction in number of the chromosomes. In certain cases Strasburger thinks that the figures show an attempt at reduction at this time without union really taking place.

The preceding evidence appears to show that a contraction figure may appear that resembles synapsis as far as one may judge from the general appearance alone, but which does not lead to conjugation of the chromosomes. We have then the alternatives of denying that here the contraction figure represents a true synapsis stage, or else of admitting that the contraction figure in itself is not a true criterion as to whether conjugation is taking place. There is also another possibility, namely, that during the contraction phase conjugation of the chromo-

somes takes place, but that the members of each pair separate before the chromosomes emerge. There is, however, no evidence at present to show that conjugation does take place.

It has been suggested by several writers that parthenogenesis itself is due to the failure of the chromosomes to conjugate, but such a view is in contradiction to the fact that in the egg of the bee that produces a male and in the male-producing egg of *Hydatina* reduction occurs and two polar bodies are formed, and still the egg develops parthenogenetically. Furthermore, in artificial parthenogenesis it has been shown, first by Wilson, later by others, that the embryo develops with half the full number of chromosomes. Nevertheless, the fact remains that in cyclical forms, where there is a parthenogenetic phase, the full number of chromosomes is present, at least in forms that have adopted this method as a means of propagation. There is no *a priori* reason that we can give as to why a race might not develop and perpetuate itself with the haploid number of chromosomes, except that it would be made up only of males, because of the presence of a single sex chromosome. For, in the only cases where an animal develops with the haploid number of chromosomes (namely, the bee and Rotifer) the individual is a male.

#### THE LIFE HISTORIES OF CERTAIN APHIDS IN RELATION TO PREDETERMINATION OF SEX

In contrast to the life cycle of the phylloxerans of the hickories the life cycles of most species of aphids is longer and in a sense open, i.e., an indefinite series of parthenogenetic forms may occur, provided certain favorable external conditions persist. The open phase of the life cycle lies between the stem-mother and the sexuparae that bear the sexual males and females.

The most important question in the aphids is whether there are two lines starting with two kinds of mothers, as in *Phylloxera caryaecaulis*, or only one line that splits later into the two sexual types. Despite the extensive literature on the life cycle of the aphids and their allies I can find only a few records that give the necessary data to decide the question whether two or one line exists. If it were found that certain females produce sexual fe-

males and other females produce males the fact would be in favor of two lines, although this is not necessarily the conclusion to be drawn from the evidence. On the other hand, if from a single female both sexual females and males arise the single line theory will cover the case. In fact, there are a few definite records where a single parthenogenetic female has been observed to give rise to sexual females, males and parthenogenetic females. Balbiani recorded in 1884 in the phylloxeran of the oak and of the grape the birth of parthenogenetic females and males, of parthenogenetic females, of males and of sexual females, and of males and sexual females from single females.

Hunter ('00) gives a number of cases where an individual produces parthenogenetic young (agamic) and sexual females, other records where an individual produces parthenogenetic young and males, and in one record all three forms were given. He found in those generations where sexual forms are produced that 66 per cent of the offspring are agamic, that about 17 per cent of the individuals are intermediate in character, i.e., they show some of the characteristics of the parthenogenetic female and some of the sexual females; that only 2 per cent of the individuals born are males and 2 per cent are sexual females. The intermediate forms belong, he thinks, to the sexual generation, i.e., they are not transitional forms in the sense that they belong to a generation preceding the sexual generation. Their occurrence is a point of great interest, for it seems to show that the difference that separates the parthenogenetic from the sexual generation is not absolutely marked off, and this would be expected if environmental rather than internal factors bring about the change.

Webster and Phillips in their government report entitled "The spring grain aphid or green bug, *Toxoptera graminum*," give many records of the output of single individuals. They show that one agamic female may give birth to males and parthenogenetic females or to males and sexual females. Among 'aberrant individuals' they record two cases where they found in a single female true eggs (i.e., sexual eggs) and living embryos (i.e., parthenogenetic embryos). Such an individual is reproduc-

ing, both sexually and by parthenogenesis. They state that Mr. C. N. Ainslie found similar cases.

Stevens has given a brief account of several forms in which the same female individual produced both males and sexual females (*Science*, vol. 26, Aug. '07). The color relations between the mother and her young are also noted. The facts recorded are highly interesting since they suggest that there is here a case of alternative color inheritance. In some cases the color appears to be due to the sex of the individual, i.e., a specific effect is produced by the combination that gives a male. But in other cases the results do not appear to conform to this relation, nor do they seem explicable by the assumption that sex linked factors are involved. They do suggest, however, as Miss Stevens pointed out, that a condition of heterozygosis in regard to the color factors may exist, and if so, chromosomes other than the sex chromosomes must be involved. But until further experimental work has been done, as Miss Stevens herself had planned, the inheritance of color in relation to sex inheritance is obscure.

In the light of these results the single line theory seems to be the most probable one for these species of aphids. The cytological work on the aphids has shown that there are but two sex chromosomes in the female, i.e., the sex chromosomes are not doubled, as in the phylloxerans, and without this doubling it is not possible, at present, to suggest a way in which the two-line scheme would work out.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

- a* to *f* Anaphase stages of the polar spindle of the egg of *Phylloxera fallax*.  
*g* Anaphase of polar spindle of male-producing egg of *Phylloxera fallax*.  
*h, i, j* Polar body of male-producing egg of same.  
*k* Female-producing egg of *P. fallax* with part of polar body nucleus and entire egg nucleus.  
*l* Polar equatorial plate of *P. fallax*.  
*m* Anaphase of polar spindle of stem mother's egg of *P. caryaecaulis*.  
*n* Anaphase of polar spindle of female-producing egg of *P. caryaecaulis*.  
*o* Anaphase of polar spindle of male-producing egg of *P. caryaecaulis* (previously figured).

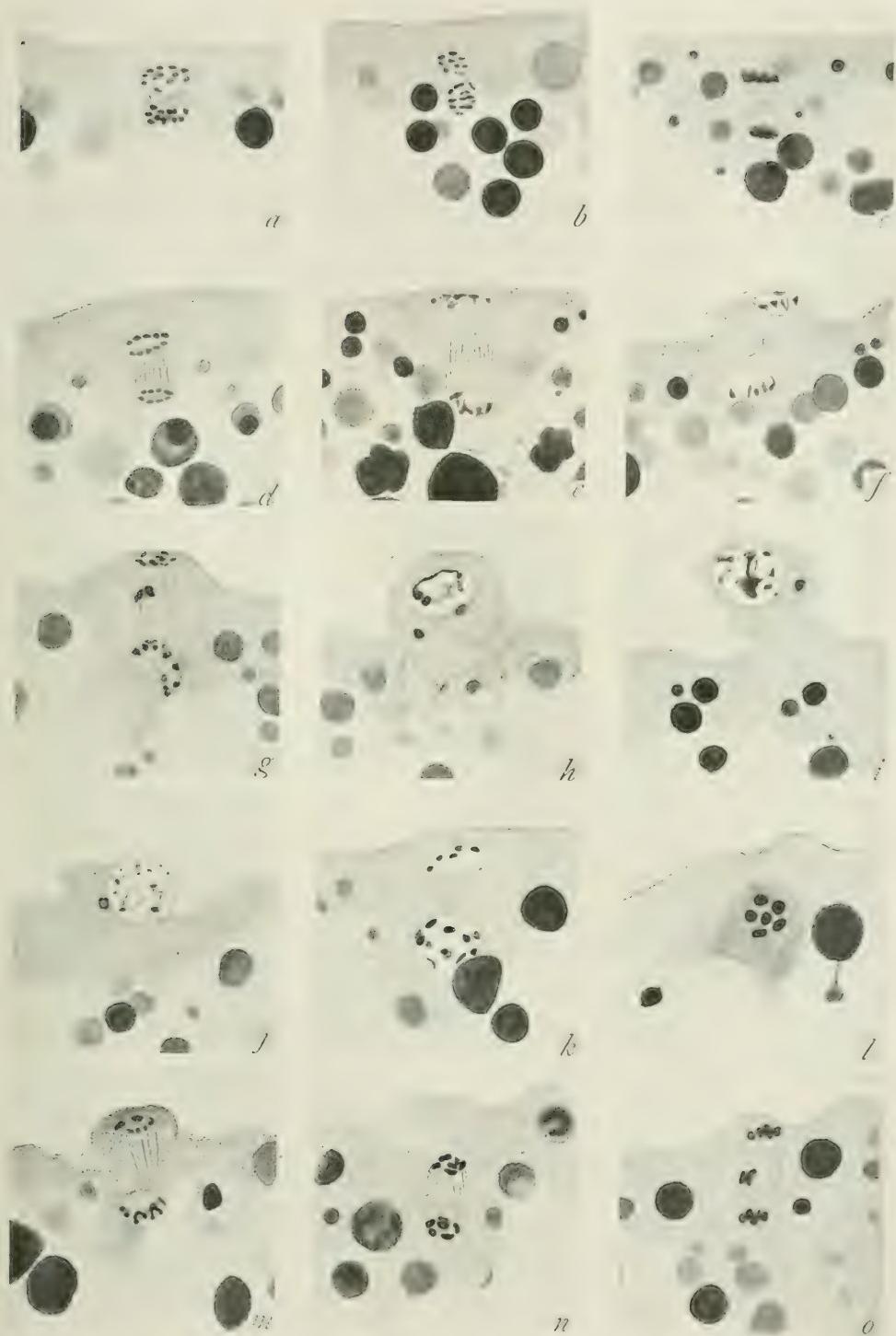


PLATE 2

EXPLANATION OF FIGURES

1 to 20 Stages in the normal spermatogenesis of the bearberry aphid, *Phylaphis cowenii*.

21 to 30 Spermatocyte division figures of ten cells in a tetraploid cyst of the bearberry aphid.





# THE EFFECTS OF THE BETA AND GAMMA RAYS OF RADIUM ON PROTOPLASM

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TWENTY-FIVE FIGURES (THREE PLATES)

The present investigation has been carried on for the purpose of determining the effects of the beta and gamma rays of radium on protoplasm. Much work has been done during the last ten years on the general effects of the radiations, but the results have been conflicting, and the opinions as to their meaning far from unanimous. Conflicting results are found to occur for two reasons; first, because different types of cells react very differently to the same stimulus; and second, because very different methods of applying the stimulus have been employed on the same kinds of cells. The first point calls for further inquiry as to why cells differ from each other in their ability to absorb the rays emitted by radium; the second, for a more careful analysis of the action of the different kinds of rays. This paper deals with the second point.

The three types of radiations given off by radium differ markedly from each other in their physical properties.

The alpha rays, which are chemically the most active, possess so slight a power of penetration that they do not reach the object of study, being entirely absorbed by the glass tube in which the radium salt is held. They may therefore be left out of this discussion.

The beta rays consist of negatively charged particles which can be deflected in a strong magnetic field. The rays are not homogeneous but are made up of particles whose speed varies from 0.3 to 0.99 of the velocity of light. The slower particles are deviated more sharply in the magnetic field than are the high speed particles and are much more readily absorbed by

matter. The latter are all absorbed by 2 mm. of lead, or by 250 cm. of air. Thus it is seen that the distance of the object to be exposed from the radium is a large factor in determining the intensity of the radiation. The slower beta particles are easily absorbed by thin layers of matter much less dense than lead. A thick mica screen is sufficient to stop them, although they pass through a very thin screen of mica without much loss of energy. Chemically, the beta rays are much less active than the alpha rays, since they are not absorbed as readily. It is therefore apparent that the slower beta rays are more active than the more rapid ones since they are absorbed in passing through matter. This point is of importance in conducting an experiment for if these more active rays are unable to reach the object of experiment the effects produced may be very different from those obtained when all of the beta rays are available.

The gamma rays are similar to the hard X-rays in many respects, but they travel with much greater velocity. For this reason they are not absorbed to any extent and their effects are of a different order from those produced by the beta rays. In a magnetic field they are not deflected. When they pass through lead of considerable thickness they are transformed into secondary beta rays, similar to the beta rays emitted by radium itself. These rays have been shown by Congdon to produce definite effects on living matter.

#### REVIEW OF LITERATURE

Up to the present time very few studies have been made on the effectiveness of the different kinds of rays, or on the relative effects of the slow and rapid beta rays. Guilleminot ('07) showed that the beta rays are more effective than X-rays when their luminescent effects are approximately the same. A more thorough study is that of Congdon ('12) in which he analyzed the effects of the primary and secondary beta rays on seedlings. He states that the gamma rays from 8 mg. of radium bromide produce no appreciable effect. Of the beta rays, the slow electrons are more effective in retarding growth than are the rapid electrons.

Abbe ('14) exposed wheat grains to mixed beta and gamma rays for varying periods and at varying distances. "The universal effect was a depression of growth exactly in proportion to both time and distance. The greatest destruction of seed life was at one inch." In no instances was there any evidence of an acceleration in the rate of growth. Carrel (quoted in Abbe's paper) found that the gamma rays produce no effect on the rate of cell growth *in vitro* but that the beta rays bring about a retardation of 25 to 50 per cent. There was no morphological change in the cells. The effect of a short radiation persisted through twenty cell generations.

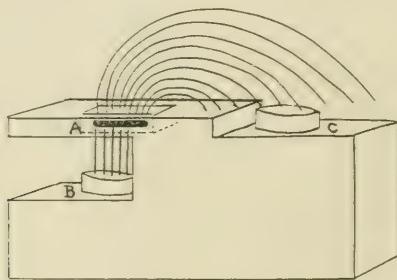
Experiments in which there has been no attempt to differentiate between the action of the different kinds of radiation have been numerous. I will not attempt to review them here for they have been mentioned in a previous paper and by Hertwig ('10) and Richards ('14). The point which is of interest in connection with the present study is that a strong radiation retards development and may produce many abnormalities. A very weak and short exposure brings about an acceleration (Congdon '12). Between these extremes it is possible to radiate developing embryos so that no abnormality results although there is a marked retardation.

#### METHODS

The beta rays can be separated from the gamma rays in a magnetic field, since the former are deviable and the latter are not. The device for separating the rays is shown in text figure A. The block is made of solid lead. The capsule containing the radium (50 mg. of the pure bromide) rests in the chamber A, the bottom of which consists of a sheet of lead 2 mm. in thickness. This is sufficient to absorb all of the beta rays projected downwards on the shelf B. Thus only gamma rays can fall on material placed in that position. When the device is placed between the poles of a strong electromagnet the beta rays are deflected in the manner indicated and fall upon material placed on the shelf C. The path of the rays, which under these conditions

is about 50 mm. may be strikingly demonstrated in a dark room by holding a willemite screen where the rays may fall upon it. The gamma rays may be similarly shown falling on the shelf B. The luminescence of the screen is about the same on both shelves. Secondary beta rays are probably produced in the lead and fall upon the shelf B but the effects they produced, as distinguished from those of the gamma rays, was not studied.

The material to be radiated was placed in small glass cells, open on top, and provided with mica bottoms. The cells were placed on the shelves B and C. In some experiments the cell



Text figure A

was placed directly above the radium capsule and at varying distances from it. The material was thus exposed to both beta and gamma rays. By varying the thickness of the mica bottoms of the cells it was possible to screen out the slower beta rays or to utilize them all. With a thickness of 0.1 mm. of mica it was found that very few of the slow beta rays were absorbed. A thickness of 0.10 mm. of mica is sufficient to screen out the slower rays, so that only the more rapid ones could affect the material.

These experiments were made at Woods Hole during the summer of 1914. For the preparation of radium and for the electromagnet with the lead device for holding the radium I am deeply indebted to Dr. Robert Abbe of New York City. I take pleasure here in expressing to him my hearty thanks.

## MATERIAL

The eggs of *Nereis limbata* and *Arbacia punctulata* were used almost exclusively in these experiments. Some work was also done on *Drosophila ampelophila* and on *Paramoecium caudatum*.

## OBSERVATIONS ON LIVING MATERIAL

*a. The gamma rays*

With the apparatus employed it is difficult to determine what percentage of the radiations falling on the shelf B is in the form of gamma rays and what is in the form of secondary beta rays. But whatever may have been their nature it is evident that they produce a slight acceleration in the rate of cell division. This is most marked in the eggs of the sea urchin, which were exposed either before fertilization or immediately afterward. Text figure 2 indicates the change in division rate. It is seen that the first indication of cell division appears about 15 minutes before a similar change occurs in the control. This difference is continued in the second cleavage. There is no abnormality in the mode of division and the larvae develop with perfect regularity. The acceleration is not cumulative; the eggs do not divide at shorter and shorter intervals. After a few divisions it is impossible to say whether the acceleration persists or not.

*Nereis* eggs do not respond at all to the gamma rays.

The eggs and larvae of *Drosophila* are not affected appreciably by the gamma rays. The rate of growth is not changed and the adults are fertile.

*Paramoecium* is not affected even by long exposures. Indeed I have not been able to see any sign of abnormality or change in the rate of division even after prolonged radiation with both beta and gamma rays. This has been the general result reached by other investigators on *Paramoecium*.

In order to test the effect of the gamma rays acting at a distance I placed the glass cells holding the material 5 cm. above the radium. When the electro-magnet was in operation the beta rays were entirely deflected so that only pure gamma rays

reached the objects. All experiments of this sort were negative, showing that the gamma rays in passing through even a short layer of air, lose much of their effectiveness.

*b. The beta rays*

*I. Experiments on Nereis.* The effect of the rapid beta rays, acting at about 50 mm. distance, is seen in a change both in the division rate and in the physical properties of the protoplasm. After the first cleavage the blastomeres divide at a slower rate than normal, although the mode of division is perfectly regular. The swimming trochophores are normal and as active as the controls, but are always behind the controls in their stage of development. The first cleavage occurs before the control eggs divide, but this apparent acceleration is due to the weakening of the peripheral protoplasm of the egg and not to an acceleration of metabolic processes.

When unfertilized eggs are placed directly above the radium, at a distance of 4 mm. the effect is far different. If the rapid beta rays are used alone, the peripheral layers of protoplasm are chiefly affected. If both rapid and slow beta rays are used the egg quickly dies, usually before the first cleavage. In the first case there is a profound change in the mode of extrusion of the egg jelly.

The normal extrusion of jelly, which has been described by Lillie ('11), takes place as follows: As soon as a sperm becomes implanted in the vitelline membrane the jelly, which has been held in delicate alveoli in the cortical layer of the egg, begins to pass through the membrane forming a thick layer outside of the egg. The alveoli, which have thus been emptied of their contents, later become filled with water so that they almost disappear from view. But before this occurs their walls may be seen extending radially out to the membrane of the egg.

In the radiated eggs no change can be noted before insemination. The oil droplets and the mitochondria, which can be seen by dark field illumination, are normal. After insemination the jelly is given off, but it has not the usual sticky character,

for the eggs do not tend to stick together as in the controls. In a few minutes the vitelline membrane is pushed away from the surface of the egg so that the perivitelline space increases in width. Figure 1, which was drawn from the living egg just before the first cleavage, shows the extent to which this increase may occur. The walls of the alveoli are drawn out so that they extend from the egg protoplasm up to the delicate plasma membrane which lies just beneath the vitelline membrane. The following measurements taken from many living eggs just before the first cleavage and 16 hours after show the extent of this increase in width:

	DIAMETER OF EGG	DIAMETER OF MEMBRANE
Normal . . . . .	87 x 100 $\mu$	87 x 100 $\mu$
Unfertilized eggs radiated for 90 min.; measured just before cleavage . . . . .	90 X 96 $\mu$	116 X 128 $\mu$
Same eggs 18 hours after insemination . . . . .	90 X 96 $\mu$	154 X 168 $\mu$

Through the kindness of Dr. G. L. Kite who dissected a number of eggs from the same lot from which these measurements were taken, I was able to observe that the physical properties of the protoplasm and of the egg membranes are greatly altered. The membranes are still sufficiently tough to hold together when the dissecting needle is pressed against them, but they are softer than normal and can be punctured without difficulty. The protoplasm, instead of being a fairly firm gel is soft, and flows freely through a small tear in the membranes. The perivitelline space is filled with a semi-gelatinous substance which stains with various protoplasmic dyes. The fact that this is more fluid than the normal jelly may indicate that it is a mixture of jelly and water. Certainly not all of the jelly is given off after insemination. The treatment with the beta rays has so altered the membranes that they are no longer able to allow the jelly to pass through them in a normal fashion. According to Dr.

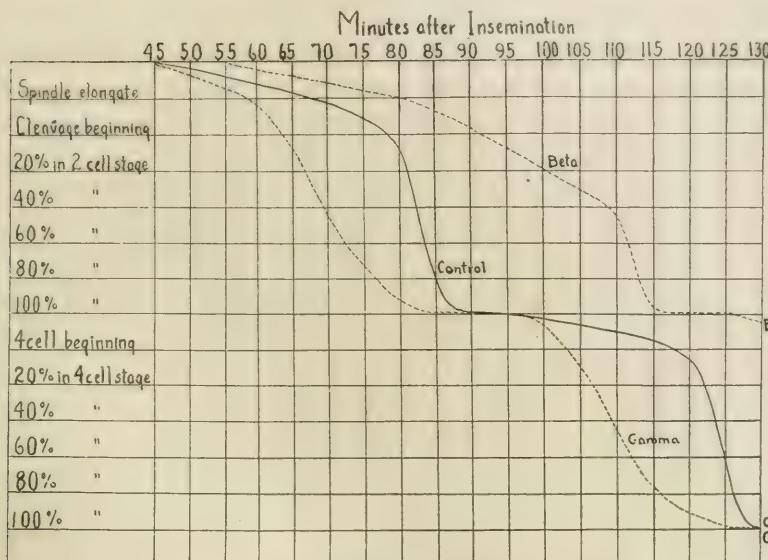
Kite the protoplasm has increased its water holding power so that the whole egg becomes soft and fluid. A further evidence of the softened character of the protoplasm is shown in figure 2. The peripheral layer is pulled in abnormally under the influence of the aster.

Cleavage occurs in a fair proportion of these eggs, but it is abnormal. After several divisions the embryo resembles a heap of shrunken cells. Occasionally the cells put out cilia.

Eggs which are treated with both slow and rapid beta rays for varying periods before insemination do not show so marked an increase in the width of the perivitelline space. But evidently they have been greatly injured since in most cases division is entirely inhibited. Those eggs which divide at all do so in a very abnormal manner.

These facts indicate that the rapid beta rays acting at a distance of 50 mm. are not strong enough to produce any marked abnormality in development, but cause a pronounced retardation in the rate of development. Acting at 4 mm. distance they affect chiefly the peripheral layers of protoplasm. When both slow and rapid rays are used cell division is usually inhibited altogether.

2. *Experiments on Arbacia.* In the experiments on the sea urchin I used both fertilized and unfertilized eggs, observing the proper precautions to have all the conditions for growth as favorable as possible. The general effect of the rapid beta rays, acting at a distance of 50 mm. is a retardation, the slowing down commencing almost at once in the case of the fertilized eggs. In text figure B the amount of retardation is shown. The curves were made from an average of many observations made on different lots of eggs. In each case the eggs were exposed for 40 minutes immediately after insemination. It will be seen that the control eggs in these experiments begin to divide about 75 minutes after insemination, and that all are in the two cell stage about 10 minutes after they begin to divide. The radiated eggs are much retarded both in beginning to divide and in attaining the complete two cell stage. At the latter time the controls have begun to divide for the second time. The pause



Text fig. B. "Sea-urchin eggs radiated for forty minutes after insemination."

between cleavages is evidently increased in length in later development since after several hours the embryos were far behind the controls. These embryos are not abnormal except in the fact that they are behind the controls. The retarding effect of the beta rays, even after so short an exposure as 40 minutes is therefore permanent, even when the embryos are placed in the best possible environment.

Unfertilized eggs respond in the same way except that a longer exposure is necessary in order to obtain the same results.

When unfertilized eggs are treated with the slow and rapid beta rays for 30 minutes or more and then inseminated with fresh sperm they throw off a very slight fertilization membrane. Cleavage is much delayed and almost always irregular. The few embryos which survive for a day show many abnormalities. Most of them never pass through the gastrula stage, and those that do, later show abnormalities of the types familiar to all who have observed the development of *Arbacia*. A careful de-

scription of such typical abnormalities has been given by Tennant ('10). It is evident from both series of experiments that protoplasmic changes in *Arbacia* are very small, if indeed they occur at all.

3. *Experiments on Drosophila.* The larvae and pupae of *Drosophila* do not show any external change even after an intense radiation for one hour. It is probable that the rate of development is somewhat retarded, although there is not sufficient evidence to prove this point conclusively. When the pupae hatch out, the flies are normally active but are completely sterile *inter se* and with normal wild stock. The sterility is however, only temporary for after about three weeks the flies become fertile again. The offspring appeared to be normal. Apparently only those germ cells which were in advanced stages of development were destroyed, while the earlier stages were merely retarded.

#### *Summary*

The gamma rays from 50 mg. of radium bromide bring about some acceleration in the rate of development of the sea urchin, but have no effect on *Nereis*. Rapid beta rays, acting at 50 mm. distance exert a retarding effect, most marked in *Arbacia*, but noticeable in *Nereis* and *Drosophila*. Acting at 4 mm. these rays affect the peripheral protoplasm of *Nereis*. When both rapid and slow beta rays are used there is a marked protoplasmic change in *Nereis* and an inhibition of development. Under the same treatment *Arbacia* shows little or no protoplasmic change, but a very abnormal development. The chief effect on *Drosophila* is a destruction of the germ cells in the later stages of gametogenesis.

#### CYTOTOLOGY OF RADIATED EGGS

In a previous paper I described a number of typical abnormalities which occur in the radiated eggs of *Nereis*. The point was made that in these eggs marked protoplasmic changes occur as a result of the treatment with small amounts of radium, and that the nuclear changes, while present, are not always obvious.

In this respect the results differ from those of Paula Hertwig ('11) who states that in *Ascaris* the only effect of radiation is on the nucleus. With larger amounts of radium I have repeated the previous experiments, and am able to confirm my former statements in regard to *Nereis*. Experiments on the sea urchin tend to confirm the observation of Miss Hertwig. This situation illustrates the difficulty of making any general statement on the effect of radium on protoplasm. In the following section I will describe the changes brought about in the eggs of *Nereis* and *Arbacia* by means of the rapid and slow beta rays.

### *I. Experiments on Nereis*

The eggs develop in different ways depending on the nature of the rays employed and on the length of exposure. In general there are three fairly distinct types of development. The first type is seen in those eggs which have been exposed to the rapid beta rays at 50 mm. distance before insemination. The perivitelline space is not increased in width; the sperm enters much earlier than normal, and the maturation divisions, which are normal, take place at a correspondingly earlier time. About 75 per cent of the eggs thus treated show this peculiarity. The second type predominates when the unfertilized eggs are exposed to the slow and rapid beta rays at 4 mm. from the source. The perivitelline space is at first normal but after a short time increases notably in width. The sperm enters early, as in the preceding case, but the maturation divisions are abnormal. Almost every egg is thus affected. The third type is seen when the eggs are exposed to the rapid beta rays acting at 4 mm. distance. The perivitelline space, in about 75 per cent of the eggs, is greatly extended; the sperm usually fails to enter, and the maturation divisions are abnormal.

The early entrance of the sperm is a very striking and constant phenomenon. In table 1, which is a summary of several experiments performed in different ways, the rate of entrance and the subsequent phenomena of maturation and cleavage are shown. The first column shows the normal course of develop-

ment. Variations from this rate are small if the temperature remains constant. It will be seen that the sperm remains external to the egg during the prophase and the early formation of the first maturation spindle, and does not begin to enter until the time of the first anaphase. At that time it is drawn through the membrane, a process which occupies less than 5 minutes. It continues its course inward during the first telophase, and is

TABLE I  
*Showing the development of Nereis-eggs radiated before insemination*

MINUTES AFTER INSEMINATION	NORMAL	RAPID BETA AT 50 MM., 75 MIN. EXPOSURE	RAPID AND SLOW BETA AT 4 MM., 90 MIN. EXPOSURE	RAPID BETA AT 4 MM., 90 MIN. EXPOSURE
10	Prophase	Prophase	Prophase	Prophase
20	Prophase	Prophase; peri-vitelline space normal	Prophase; peri-vitelline space normal	Prophase; peri-vitelline space wide
30	1st metaphase at periphery	sperm entering; 1st anaphase	1st metaphase	1st metaphase
35	same; sperm still external	1st polar body extruded	sperm entering; anaphase	
40	sperm entering; 1st anaphase	sperm in center of egg; 2nd metaphase	sperm entering; 1st anaphase; perivitelline space wide	Very abnormal development with suppression of the 1st polar body; in many cases the sperm does not enter
45	1st anaphase and telophase	2nd anaphase and telophase	sperm in center of egg; abnormal polar divisions	
55	sperm in center of egg; 2nd metaphase	fusion of pronuclei	a great variety of abnormalities	
65	fusion of pronuclei	cleavage	cleavage rare	cleavage rare and abnormal
75	cleavage			

in the center of the egg when the metaphase of the second spindle has formed. The pronuclei fuse about 65 minutes after insemination.

The second column shows the rate at which these phenomena occur in unfertilized eggs which have been exposed to the rapid beta rays acting at a distance of 50 mm. The perivitelline space is not appreciably widened. The sperm enters about thirty minutes after insemination, that is, about 15 minutes ahead of the control. In the meantime the first anaphase has developed. In other words, the development of the egg has kept pace with the early entrance of the sperm. By the time the sperm is in the center of the egg (in 40 min.) the first polar body has been extruded and the second metaphase figure has developed. Here again, the whole development occurs at exactly the same rate as in the controls. If the living eggs only had been observed the conclusion might have been drawn that the treatment with radium stimulated the eggs to divide at a faster rate than normal. But the cytological evidence just presented shows that there has been no stimulation. The weakened egg membranes have permitted the sperm to be drawn in earlier than usual, but that is all. There are no constant abnormalities to be found in these eggs either during the maturation divisions or during cleavage. The subsequent development is normal, but the larvae develop at a slower rate than the control animals. The effect of these rays under these conditions is therefore to weaken the egg membranes and to bring about a retardation in the growth of the embryo.

The effect of the slow and rapid beta rays is shown in the third column. The perivitelline space, which at first is of normal width, increases later in a striking manner which has already been described. The protoplasm at the periphery of the egg is much changed in appearance. The finely granular character seen in normal eggs, is lacking, and in its place is a fairly homogeneous substance in which are held numerous faintly staining spherules (fig. 3). Occasionally it presents a vacuolated appearance seen in figure 4. As the perivitelline space increases in width the entrance cone is also pulled out so that it stretches

across the entire space and reaches the membrane just below the sperm (fig. 4). The sperm enters somewhat earlier than in the controls. The cone does not sink back into the egg protoplasm but remains elevated and the sperm penetrates throughout its entire length (fig. 5). In some instances the sperm does not enter at all.

The egg develops normally as far as the first metaphase. In some instances development up to cleavage is normal, but such eggs do not develop far, for they die before reaching the trochophore stage. Of the abnormally developing eggs about 20 per cent show a curiously small polar spindle. The whole figure is crowded to the periphery of the egg. The polar body, however, is normally extruded and the second maturation spindle is apparently normal. In about 40 per cent of the eggs the centrosomes of the first polar spindle divide to form a tripolar or multipolar spindle (figs. 6 and 7). The chromosomes are perfectly normal and the asters are well developed. But in such cases the first polar body is not extruded. The chromosomes remain in the condensed condition for a considerable period, after which, those nearest the periphery are extruded in the second polar body. The reason for believing that this is the second and not the first polar body is that immediately after its extrusion the chromosomes become vesicular, just as they do in normal eggs after the second polar body has been given off. The mechanism involved in the extrusion of those chromosomes is not clear since in no case have I been able to find any spindles. Either the stage during which they are present has been passed through very quickly, or else the fibers do not stain; figures 8 and 9 show this condition. In figure 8 only a few chromosomal vesicles are shown. The polar body is unusually large, and contains little chromatin. Figure 9 shows a later stage. The polar body has formed completely and the remaining chromosomal vesicles, whose position indicates that they were lying in a tripolar spindle, are now distinct. There are about 28 karyomeres in the vesicles.

If the sperm fails to enter, development proceeds in a very different way. As before, multipolar spindles form at the first maturation division. The first polar body is suppressed, and

the chromosomes, following the extrusion of the second polar body, develop into large chromosomal vesicles. Such an egg is shown in figure 10. The sperm is seen still exterior to the egg membrane which is not lifted far off from the egg. There are at least 40 vesicles present in the entire egg and the karyomeres number about 250. Such a number of vesicles is probably due to more than one division of the chromosomes at the first metaphase. The karyomeres may have fragmented by direct division. Subsequently they have grown, since each one is fully as large as those found in normal eggs.

The third type of development is seen in eggs radiated with the rapid beta rays at 4 mm. distance. The increase in the width of the perivitelline space is much more marked than in the preceding type, for it appears earlier and is greater in extent. More than 75 per cent of the eggs show such an abnormality. In the great majority of cases the sperm does not enter at all, and can be found more than an hour after insemination still outside of the egg. This phenomenon may be due to the fact that the vitelline membrane is so rapidly pushed away from the egg that no fertilization cone could extend far enough out from the egg to reach it (fig. 11).

The development of these eggs proceeds normally until the time when the sperm should enter, that is, through the first metaphase. The chromosomes are not extruded, and no first polar body forms at all. The inner centrosome of the spindle divides forming well marked tripolar and multipolar spindles (figs. 11 and 12). Figure 13 shows a small protoplasmic protuberance at the point where the polar body should be given off. This is a very common phenomenon. The chromosomes which are very numerous, owing to the multipolar divisions, now become vesicular, just as they do in normal eggs at the end of the second polar division. In the meantime the second polar body has been extruded although the method, as in the preceding case, is obscure. I could not determine how many chromosomes are extruded at this time, but from indirect evidence I believe the number to be 14, that is, the haploid number. The remaining chromosomes, of which there may be 28 or 42 or even more,

depending on the number of divisions of the first polar chromosomes, now migrate, still distinct, to the center of the egg, or they may grow in size until they finally fill a large portion of the egg. The latter condition is seen in figure 13. In the former case (and such instances are rare) the egg divides abnormally. The chromosomal vesicles become arranged on the cleavage spindle and are unequally divided in the two daughter cells. The larger number stays in the larger blastomere. The asters are very slightly developed, but the spindle fibers and interzonal fibers are very obvious; figure 14 shows this point. The total number of vesicles remaining in the larger blastomere is about 28, and in the smaller, about 14. Whether these numbers are significant or only accidental cannot be said since there are so few cases of this phenomenon.

Many of these eggs extrude no polar body at all. In such instances the chromosomal vesicles fuse together and the karyomeres spin out into a spireme somewhat similar to that seen in normal eggs immediately after the germ nuclei have fused. But in these nuclei there is no nuclear wall (fig. 15) the chromosome lying in a vacuole filled with a very faintly staining substance.

These observations point to the conclusion that cells may be stimulated or retarded without suffering any marked morphological injuries. The effect has been physiological since only the rate of metabolism has been affected. But with more severe radiation the retardation is not so apparent because the embryos die before developing far. A comparison of the injuries brought about by the slow and rapid beta rays acting together with those induced by the rapid rays alone reveals the curious fact that the more intense radiation occasions less apparent disturbances. This phenomenon superficially resembles that described by Hertwig, who found that when the unfertilized frog egg is radiated intensely, development after insemination is more normal than when it is radiated more moderately. His explanation is that the egg nucleus has been entirely inhibited from taking part in cell division, so that only the normal sperm nucleus divides. But in *Nereis* development after intense radiation is

never haploid, and the egg nucleus always develops, though abnormally, through the maturation periods. It is impossible to inhibit completely the activity of the egg nucleus without destroying the entire egg.

*2. Experiments on Arbacia.* A cytological study of *Arbacia* eggs, radiated both before and after insemination reveals the fact that the treatment produces very slight effects on the cell constituents. Clear cut abnormalities such as were abundant in *Nereis* are here very rare. Indeed it is only by careful search that they can be found. This does not signify that radium is incapable of effecting marked cytological changes in sea urchin eggs, but merely that the treatment given in these experiments was not severe enough. But inasmuch as abnormal development always follows prolonged radiation, it is evident that profound changes have taken place which cannot be rendered visible by the technical means now at hand. The amount of visible injury cannot be considered an index to the actual condition of the cell constituents.

The eggs were exposed for varying times to the gamma rays, the rapid beta rays, and to a mixture of the rapid and slow type. In those eggs treated with the gamma rays there is no sign whatever of injury. As stated before, the only effect of such a treatment is seen in the slight acceleration of cell division. Exposure to the rapid beta rays likewise produces no visible cytological changes, but only a marked retardation in the rate of development. When both slow and rapid beta rays are utilized some effect on the cell constituents can be seen.

Unfertilized eggs were exposed for 50 to 60 minutes to all the available beta rays, after which they were inseminated in finger bowls. In each experiment a parallel series of exposures was made on a very few eggs. Such a control is necessary since overcrowding of the eggs frequently produces the same abnormalities as the radium. By having two controls for each experiment the danger of drawing false conclusions was minimized. In the experiments to be cited there was always a sharp distinction between the behavior of the radiated and the control eggs.

The entrance of the sperm is normal in every case, and polyspermy is as rare as in the controls. As the sperm penetrates the peripheral protoplasm of the egg it revolves, and an aster develops in front of it (fig. 16). The further course of the sperm is not marked by any abnormalities (fig. 17). In about twenty minutes the sperm head becomes closely applied to the egg nucleus where it remains as a distinct cap for some time before it completely fuses with the egg nucleus. In the meantime the sperm aster divides and the daughter asters migrate to opposite sides of the cleavage nucleus (fig. 18).

Up to this time the egg nucleus is entirely normal. Before insemination it is filled with a tangle of chromatin threads suspended in a delicate linin network, a condition which persists until the sperm nucleus begins to fuse with it. The first sign of abnormal development appears at this time. Some of the chromatin of the egg nucleus condenses into deeply staining spherical bodies which are scattered throughout the nucleus. As a rule they are comparatively small (fig. 19) but may be very large (fig. 20). In this condensed condition they remain throughout subsequent development and may be seen in the anaphase of the first cleavage lagging behind the chromosomes (figs. 22 and 23).

The remaining chromatin at first spins out into very delicate threads, during which time the mingling of the parental chromatin takes place (fig. 19). This stage is followed by a gradual shortening and thickening of the threads (figs. 20 and 21). In the meantime the astral rays (not shown in the figure) grow in and become attached to the rod-like chromosomes which have resulted from the shortening of the chromatic threads. This whole process is normal in every respect.

The chromosomes can be counted during the anaphase. Many counts show that development is, without exception, diploid. The normal diploid number is 34. The appearance of the mitotic figure resembles in many ways the figures of Hertwig ('12). In his experiments on *Sphaerechinus* he radiated the sperm only, and found that the sperm chromatin breaks up into numerous masses of irregular shape which in many instances, are involved

in the spindle in which the dividing egg chromosomes are located. In my experiments, which are the reverse of his, since only the eggs are radiated, it is the egg nucleus that gives rise to the chromatin masses. That they represent abnormal chromosomes is probable, since the number of these bodies, added to the number of normal chromosomes in any figure, gives the usual diploid number. Evidently, therefore, only a portion of the egg chromatin has been injured severely enough to produce obvious changes in appearance.

The achromatic portion of the mitotic figure is normal in every respect. The further stages of division are normal except for the presence of the injured chromosomes which may lie anywhere in the spindle. Occasionally they go to the poles where they may be seen still condensed at the telophase, when the other chromosomes have already become vesicular.

This brief description indicates that the effects of a short radiation are very slight. There is little evidence that the protoplasm has been injured. A longer exposure undoubtedly would produce more marked injuries, but such an exposure is difficult to make in view of the fact that sea urchin eggs are extremely sensitive to overcrowding and to a prolonged stay in small quantities of water, conditions which are necessarily imposed during radiation.

#### DISCUSSION

The character of the response of protoplasm to radium radiations depends on the nature of the protoplasm itself, and on the intensity of the exposure. In regard to the first point little can be said except that cells differ from each other in their susceptibility, wholly apart from the fact that each cell varies in susceptibility during different phases of its own activity. It has been pointed out that an exposure of thirty minutes to the beta rays will bring about in the developing sea urchin changes which are as pronounced as those produced in *Nereis* after ninety minutes exposure to rays of the same intensity. Some Protozoa are entirely unaffected at the end of fourteen hours of exposure, while others are killed in a shorter period. There is a similar

variation in the responses of the Bacteria. Obviously, those cells which are injured contain substances which absorb the rays, while those which are uninjured allow the rays to pass through unchanged. The factors which determine the power of absorption of materials are not well known. "The absorption of beta rays is an atomic phenomenon and is not affected by the chemical combination of the atoms. Such a relation appears to hold generally for all types of radiations emitted by radioactive substances" (Rutherford). Until more is known on this point it will be impossible to predict what effect a given exposure will produce. These facts throw no light on the nature of protoplasm, but accentuate the point that the protoplasm of one type of cell differs from that of another cell.

Disregarding these differences, it may be said that a weak radiation accelerates, and a stronger one retards cell division. Acceleration is not followed by any abnormality. A careful study of this point has been made by Lazarus-Barlow and Beckton ('13) who used exceedingly small quantities of radium on Ascaris eggs. Tests made upon many thousands of eggs showed that cell division is accelerated when the exposure is not too prolonged. After an optimum length of exposure the rate of cell division is gradually retarded. I have shown that sea urchin eggs are accelerated by a weak stimulation. The kind of rays seems to make no difference with the result. Inasmuch as the gamma rays are very penetrating, and therefore are not absorbed to any extent, they are the 'weakest' and must be allowed to act for a long time before they can produce any effect. The beta rays are more readily absorbed and will produce an acceleration if not allowed to act for too long a time. If the alpha rays are allowed to act in unison with the other types, acceleration will follow after a few seconds' exposure. These rays are about one hundred times as effective as the beta rays, and the beta rays are more effective than the gamma rays in the same proportion. These figures correspond roughly to the respective powers of ionization of the rays.

Retardation follows a moderate radiation of the beta rays. This effect is not peculiar to them, for if they are mixed with

gamma rays the results are the same provided the intensity of radiation is equal. The effect is cumulative, and persists through many cell generations (Carrel '14). This is shown also in the experiments on *Nereis* and on *Arbacia*. It has also been found that a radiated cancer, in which cell division has been retarded by exposure to the beta rays, may be transplanted several times and still show the effects of the radiation. There is no appearance of abnormal development, or of any visible changes in the cell constituents. The treatment, in every case, has served merely to decrease the rate of normal metabolism without disturbing the process itself.

A very strong radiation with the beta rays (which must necessarily be mixed with the gamma rays) or with all three kinds at once, results in profound morphological changes in the cells. The type of changes thus induced varies in different cells. In the sea urchin and in *Ascaris* the nucleus is most readily affected. In *Nereis*, on the other hand, it is the protoplasm which first undergoes degenerative changes. Whether this is due directly to an ionization of the chemical compounds of protoplasm is an open question. Inorganic materials are ionized during radiation, but living matter may not be affected in the same way. A lytic action occurs, as shown in the liquifying of the protoplasm in *Nereis*, and in the breaking up of the chromatin of *Ascaris* and other forms.

These profound changes in the physical constitution of the cells is accompanied by changes in the behavior of their constituents. In this respect cells differ greatly. In *Ascaris* the achromatic portion of the mitotic figure is uninjured, while the chromatin is broken up into granules. In *Nereis*, on the contrary, the chromosomes split with great precision, but the spindles are abnormal and are sometimes entirely absent. But I have never found an egg so injured that it did not make some attempt, however abortive, to go through its usual development.

The hypotheses which have been advanced in explanation of the phenomena which follow a severe radiation were discussed in a previous paper (Packard '14). Hertwig's view, which is based on a study of forms in which only the chromatin is injured,

is that prolonged exposure may so injure the chromatin that it is unable to play a part in cell division. If the sperm alone is radiated, it merely acts as a stimulus to induce in the egg parthenogenetic development. If the egg is radiated, the sperm nucleus alone divides, the egg nucleus taking no part in subsequent development. A less severe radiation of either element serves to generate in the chromatin a poison which brings about abnormalities in growth.

I have been unable to find any evidence of parthenogenetic development either in *Nereis* or in *Arbacia*. When the eggs are radiated development is either diploid or does not occur at all. The same is true if the sperm is radiated. If the eggs or sperm are not greatly injured development is diploid but abnormal.

It is evident that no generalization on the effect of radiations can be based on the behavior of a single form, for it has been shown that there are several types of response among the cells already studied. Nor can we assume that the effect is directly on the nucleus or on the protoplasm. If it were on the former we should expect that exposure of the cells during the resting stage of the nucleus would be followed by greater abnormalities than would obtain when the radiation is made during mitosis, since in the former period the chromatin is more finely divided and presents a larger surface to the rays. But the reverse is the case. Mottram ('13) has shown that *Ascaris* eggs are eight times more susceptible during division than during the resting stage; that is, there are eight times as many deaths following an exposure made during mitosis than during the latter period. It has also been observed that cancer tissue is much more susceptible to the rays when it is growing rapidly than when it is nearly stationary. This indicates that an explanation for these phenomena must take into account the differences between the physiological state of the cell constituents during these periods.

During the resting period the interchange of material between nucleus and protoplasm is small compared with the amount which takes place during cleavage. At the latter time the

amount of oxygen which is taken in and of carbon dioxide which is given off is greatly increased. The agents concerned in the utilization of oxygen and in the production of carbon dioxide are undoubtedly the intracellular enzymes which during division are more active than during the resting period. Acceleration of the normal metabolic processes must necessarily involve a quickening of the enzyme action. In like manner, retardation of those processes is connected with a slowing down of the activities of the enzymes.

According to Gager ('08), "The broadest, and at the same time the most definite generalization warranted by the work done so far is that the rays of radium act as a stimulus to metabolism. If the stimulus ranges between minimum and optimum points, all metabolic activities, whether constructive or destructive, are accelerated; but if the stimulus increases from the optimum toward the maximum point it becomes an over-stimulus, and all metabolic activities are depressed and finally completely inhibited." The fact that enzymes may be accelerated or retarded has been shown by Richards ('14 b) who states that an exposure of two minutes to X-rays produces an acceleration in their activity, while an exposure of more than five minutes causes a retardation. If we assume that such reactions are duplicated in the living cell we have a logical explanation for the phenomena which have been described.

The results of these experiments suggest further lines for research. It has been shown that in the sea urchin the chromosomes are not all affected in a similar manner, for some are evidently injured while others are not visibly changed. Payne ('13) has pointed out that when the egg of *Ascaris* is moderately stimulated, the chromosomes show marked differences in their reaction to the treatment. After the egg has divided, it is found that the chromatin of the sex cells is noticeably different from that in the somatic cells. This indicates that the two kinds of chromosomes are physically different, as Boveri has stated. This point can be tested by studies on the reactions of the chromosomes of those bugs in which the X chromosome can be

distinguished. And should the behavior of the chromosomes in *Ascaris* find a counterpart in the behavior of the chromosomes of the bugs we would have a simple and elegant method of testing some of the hypotheses concerning the rôle of the X chromosome.

#### SUMMARY

1. Very mild radiation by means of the gamma rays from 50 mg. of radium bromide produces an acceleration in the rate of cell division in *Arbacia* without producing any abnormalities. These rays have no effect on the development of *Nereis* or *Drosophila*.
2. Moderate stimulation by means of the beta rays, obtained by separating them from the gamma rays in a strong magnetic field, brings about a retardation of growth in *Arbacia* and *Nereis*, which is followed by no abnormalities.
3. More intense radiation in which both beta and gamma rays are used, results in a liquifying of the protoplasm in the *Nereis* egg, and the development is abnormal. The eggs of *Arbacia* show no protoplasmic changes, but the chromatin is injured.
4. There is no evidence for parthenogenetic development.
5. Acceleration and retardation may be caused by a change in the rate of enzyme action brought about by the radium treatment.

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## PLATE 1

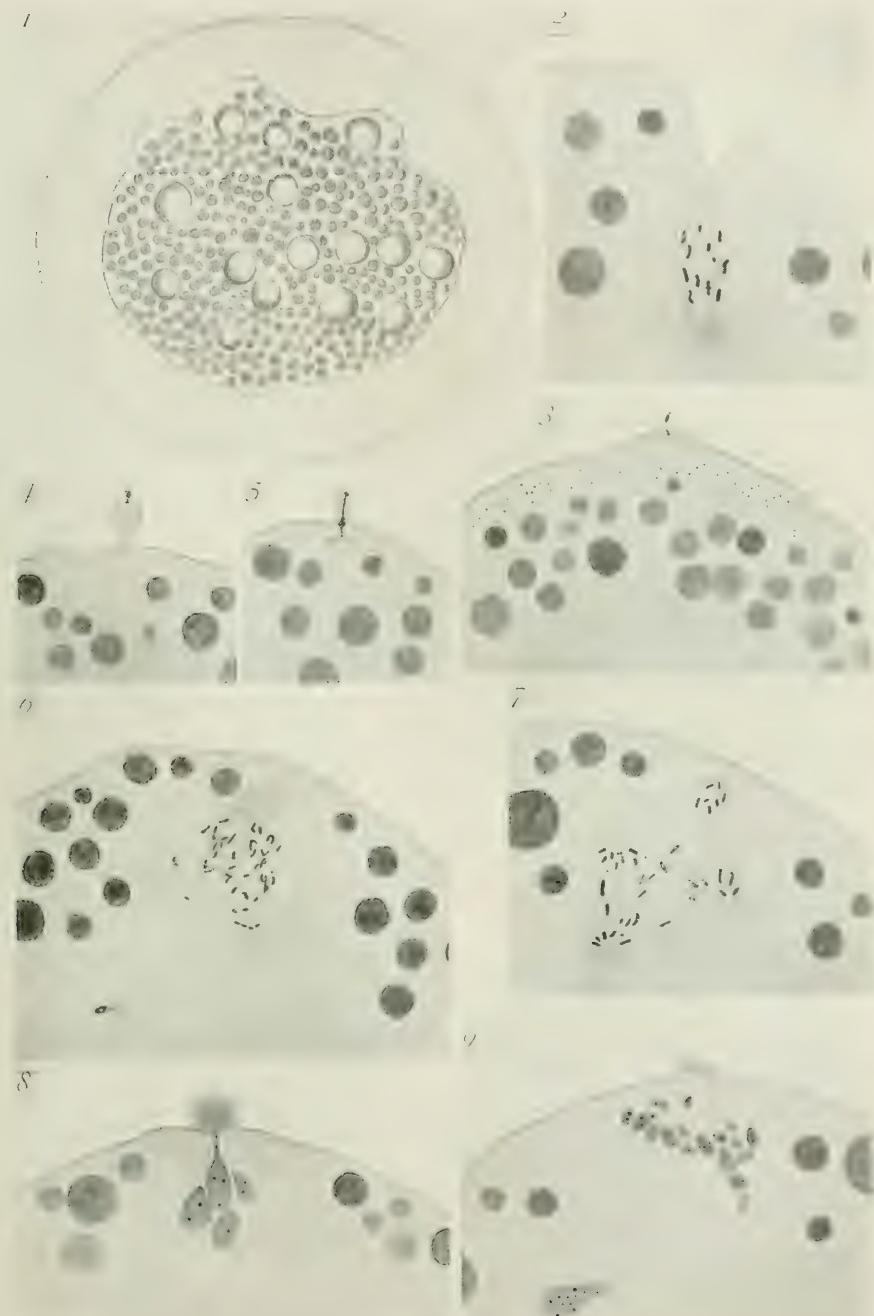
### EXPLANATION OF FIGURES

All the drawings were made with the camera lucida. Unless otherwise stated the magnification is  $\times 875$ . Figures 1 to 15 inclusive show the development of *Nereis* under the conditions described; figures 16 to 23 are of *Arbacia*.

- 1 Drawn from the living egg.  $\times 475$ . This egg had been radiated with rapid beta rays at 4 mm. for 1 hour; drawn just before cleavage. The very wide perivitelline space is traversed by delicate strands representing the walls of the alveoli.
- 2 Section of egg treated as above; 35 minutes after insemination; the softened protoplasm at the periphery has been pulled in under the influence of the aster.
- 3 Egg radiated for  $1\frac{1}{2}$  hours with both rapid and slow beta rays, 40 minutes after insemination. The protoplasm at the periphery lacks the usual granular appearance.
- 4 Same treatment, 40 minutes after insemination; the protoplasm is vacuolated.
- 5 Exposure as in fig. 1. 35 minutes after insemination; the sperm has begun to enter the egg.
- 6 Same, 45 minutes after insemination; the sperm is now in the center of the egg.
- 7 Egg exposed to the slow and rapid rays for 30 and 50 minutes after.
- 8 Same, 70 minutes after insemination.
- 9 Same, 60 minutes after insemination.

EFFECTS OF RAYS OF RADIUM ON PROTOPLASM  
CHARLES PACKARD

PLATE I



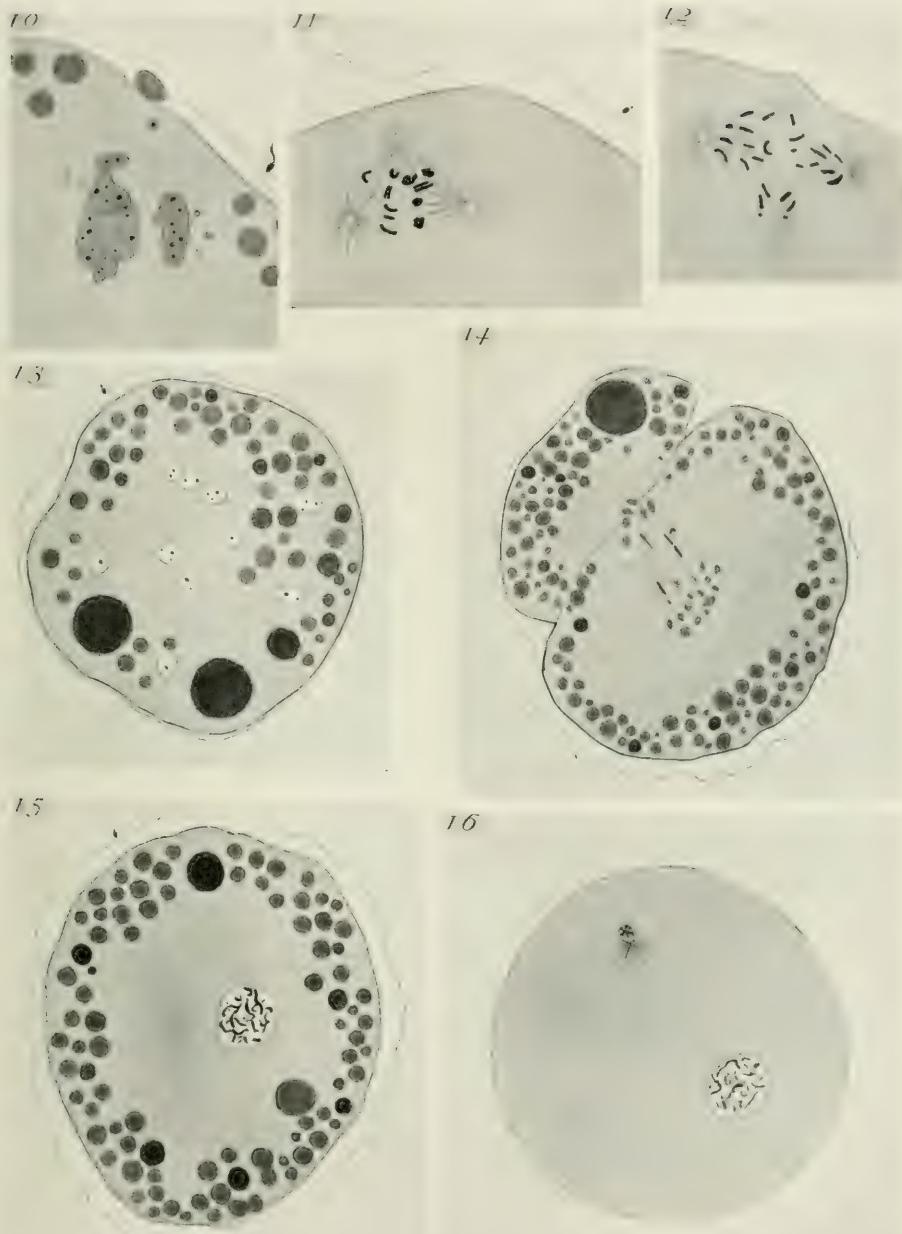
## PLATE 2

### EXPLANATION OF FIGURES

- 10 Same, 60 minutes after insemination; the sperm is still external.
- 11 Egg exposed to the rapid beta rays at 4 mm. for  $1\frac{1}{2}$  hours; the chromosomes have split in a normal fashion; 45 minutes after.
- 12 Same, 55 minutes after insemination.
- 13 Same, 55 minutes after; the whole egg is filled with these vesicles.
- 14 Same, 70 minutes after insemination; cleavage is superficially regular but the distribution of chromosomes is abnormal.
- 15 Same, 70 minutes after insemination; the sperm has not entered and no polar bodies have been given off.
- 16 to 23 Sea urchin eggs; in each case the eggs were exposed to the rapid and slow rays acting at 4 mm. distance.
- 16 Entrance of the sperm head; 20 minutes after insemination.

EFFECTS OF RAYS OF RADIUM ON PROTOPLASM  
CHARLES PACKARD

PLATE 2



### PLATE 3

#### EXPLANATION OF FIGURES

- 17 Further progress of the sperm nucleus; there is no sign of abnormality; 25 minutes after insemination.
- 18 Fusion of the germ nuclei; 30 minutes after insemination.  $\times 1700$ .
- 19 The cleavage nucleus; the chromatin is in very delicate threads; some of the chromatin is aggregated into irregular bodies; 60 minutes after insemination.  $\times 1700$ .
- 20 Later stage in the formation of the chromosomes; 60 minutes after insemination.
- 21 Just before the prophase.  $\times 1700$ .
- 22 Prophase. The injured chromatin has assumed a variety of shapes, and is irregularly distributed.  $\times 1700$ .
- 23 Anaphase of the first cleavage; the injured chromatin is lagging behind.  $\times 1700$ .

EFFECTS OF RAYS OF RADIUM ON PROTOPLASM  
CHARLES PACKARD

PLATE 3

17



18'



19



20



21



22



23





# THE EFFECTS OF CARBON DIOXIDE ON THE EGGS OF ASCARIS<sup>1</sup>

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FIFTEEN TEXT FIGURES AND THREE PLATES

The material upon which the present study is made was placed in carbon dioxide on July 14, and removed on October 9, of the same year (1913). Professor Boveri, in an attempt to keep the eggs of Ascaris for a long time without allowing them to undergo development, had a number of smears<sup>2</sup> from one female placed in a stoppered glass jar. The air of the jar was then replaced by passing a current of carbon dioxide through it for an hour and a half and, after sealing it carefully to prevent the escape of the gas, it was placed in a basement room in the Zoölogical Institute at Würzburg.

At the time the eggs were placed in the gas, no ill effects were anticipated, consequently, no control smears were preserved to show the exact nuclear condition in which the eggs were at that time; and, after they were removed from the gas, they were placed directly on ice where they remained until used. When a few smears were allowed to undergo full development later, it was found that only part of the worms were normal. Professor Boveri called my attention to the fact and placed the material at my disposal with the suggestion that I determine the cause of the abnormal development which part of the worms showed.

Although the material for this study was obtained in Würzburg, the greater part of the work has been done since my return

<sup>1</sup> A preliminary note on this subject has been published by the author (1914).

<sup>2</sup> The smears were made on ordinary microscope slides according to Boveri's well known method.

to America. I take this occasion to express my thanks to Professor Boveri for suggesting the problem and for placing the necessary material at my disposal.

In preserving the eggs, a mixture of 4 parts 95 per cent alcohol and 1 part glacial acetic acid was used. They were stained *in toto* and mounted in glycerine.

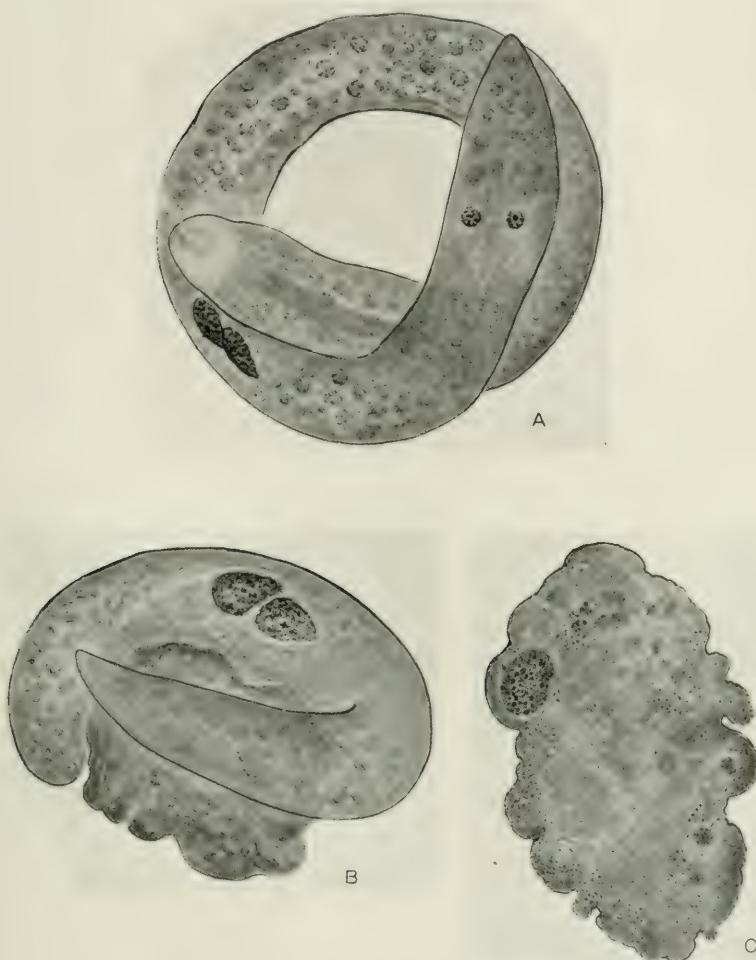
#### DESCRIPTIVE

Among the embryos which had been allowed to undergo full development, one finds perfectly normal specimens; specimens in which only one end of the body is developed; and lastly, totally disorganized embryos. The problem was primarily to determine the causes which produced the abnormalities observed, but as the work went forward a number of other questions of general interest came up which will be touched upon in the following paper.<sup>3</sup>

A drawing of a normal worm, which developed from an egg exposed for three months to carbon dioxide, is given in figure A. A blunt anterior end with the pharynx and the pointed posterior end may be seen, and, in addition, towards the posterior part of the body the large deeply-staining nuclei of the primordial germ cells. Typically there are only two of the latter but occasionally more occur; in one case six were present.

The abnormal embryos are of two general types. One of these has the posterior end of the body fully developed while the anterior end is disorganized. The second type is characterized by the absence of organization in its blastomeres. The first type of embryos is shown in figure B. This occurs in roughly 33 per cent of the embryos (in 54 cases out of 165 examined for the point). The pointed posterior end is clearly seen together with the primordial germ cells, but there is much variation in the degree to which the posterior part of the body is developed. We

<sup>3</sup> A brief description of the normal development of *Ascaris* is given on page 367. Any one not familiar with the cleavage, or with the nomenclature, in this worm, will find it helpful to read this over together with a glance at the schematic diagrams given. The nomenclature of Boveri has been used throughout the present work.



Text figures A to C

may, in rare cases, find fully seven-eighths of the worm normally formed, or we may find nothing but a pointed stump; figure B gives a fairly typical case.

An example of the second type of embryo is given in figure C. Such individuals are found in about 40 per cent of the cases (66 cases in 165). The greatest variation is noted in the appearance

of such embryos. Typically they consist of a mass of cells, among which one may distinguish the primordial germ cells, but no organization exists and quite frequently it is evident that no cleavage cavity was ever formed in the embryo, consequently, that gastrulation had not taken place.

Eggs preserved as they were taken from the ice chest, where they had been since their removal from the CO<sub>2</sub>, showed a slight amount of development. All of them had divided at least once, and something less than half (227 out of 505 eggs counted) had reached the 3-cell stage. Occasionally, in such a preparation, a 4-cell stage is found.

An examination of these 3-cell stages (fig. 1) shows that the S<sub>1</sub> blastomere has divided to form the A and B cells, while the P<sub>1</sub> blastomere is in a 'resting stage.' The nuclear condition of the A and B cells is normal: as may be seen by the figure, waste chromatin occurs in one or both of the cells showing that the diminution process has taken place, but the two cells do not always lie pressed against each other as is normally the case (compare fig. 1 with text fig. F). In 50 eggs out of 78, examined at random for this point, the A and B blastomeres were separated, in extreme cases the two cells lying on opposite sides of the P<sub>1</sub> cell.

Among the eggs in the 2-cell stage, 101 out of 278 cases showed the S<sub>1</sub> cell dividing, with the chromosomes in the equatorial plate phase. The remainder showed resting nuclei in both the S<sub>1</sub> and P<sub>1</sub> blastomeres: A close examination of the equatorial plates in the dividing cells shows an abnormal condition of the chromatin (figs. 2a to 2d). Figures 2b, 2c, and 2d are drawn at a higher magnification. In practically every case (96 out of 101 eggs examined for the point) the chromosomes were found fused together. This fusion seems to affect the ends principally (figs. 2a, 2d) leaving the middle portion free, but in extreme cases even the middle parts are involved and all four chromosomes are clumped together into one mass (figs. 2b and 2c). One very constant feature of this fusion is the formation of what appear to be vacuoles in the fused ends. It is also to be noted that when only the ends of the chromosomes are involved the middle

portions have the normal clumped or lumped appearance which precedes diminution (compare fig. 2d with fig. 9).

In rare cases, both the  $S_1$  and the  $P_1$  cells were dividing at the same time (fig. 3). When this occurred, the chromatin of the  $S_1$  blastomere showed the fused condition, while the chromosomes of the  $P_1$  cell were normal.

In the 2-cell stages with the nuclei in the resting phase, no departures from the normal could be distinguished.

When the eggs are allowed to develop a short time before they are preserved, the  $P_1$  blastomere begins to divide. The elongated chromosomes, so characteristic of the primordial germ cells, are always found and aside from the axis of division, the cell appears normal. Here and there a tendency for the four chromosomes to break up has been noted (text fig. J and L), but this is not to be regarded, I think, as an effect of the  $\text{CO}_2$ . The point will be taken up in detail later under the heading 'Anomalies.'

If the eggs are allowed to develop further until half are in the 4-cell stage, a variety of conditions are found in the 4-, 3- and 2-cell stages. Needless to say, these various conditions arise out of the 2- and 3-cell stages described above.

Among the 4-cell stages we find three different types of embryos. One of these (fig. 5) is perfectly normal, both in the position of the blastomeres and in their nuclear conditions. A second type is characterized by the failure of the four blastomeres to form a rhombus, as they normally should do (fig. 4).<sup>4</sup> Most frequently the planes connecting the two pairs of blastomeres lie at right angles to one another, but this is variable, every imaginable condition being met with in a large number of eggs. The third type is one where, in addition to an abnormal position of the blastomeres, we find an unequal distribution of the chromatin between the A and B blastomeres (fig. 6; this drawing is of a 3-cell stage, selected because it shows particularly well the

<sup>4</sup> It is not to be thought that these various positions which the A and B blastomeres occupy are just phases of the normal shifting which certain cells of the egg undergo about this period. As will be seen, these positions are retained in later cleavage.

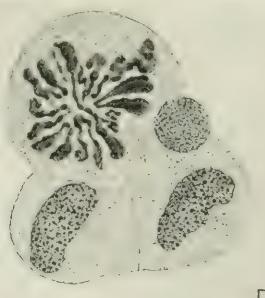
unequal distribution of the chromatin). Here we fail to find the usual waste chromatin in either the A or B cell. On the other hand, one of the cells in such eggs always contains a very large amount of deeply-staining reticular chromatin (fig. 6) while its mate lacks this. This is a very constant feature of eggs in which there has been an unequal distribution of the chromatin between the A and B blastomeres.

Among the 3-cell stages there is always a certain proportion of eggs which are perfectly normal, as far as can be determined. The  $S_1$  cell is divided, the blastomeres lie pressed up against each other, and the waste chromatin lying in the cytoplasm of these cells indicates that diminution has taken place. There is a second type of 3-cell stage which is very striking (figs. 7 and 8). In the egg shown in figure 7, the  $P_2$  and the EMSt blastomeres have separated—are in a stage of division, in fact while the  $S_1$  cell is undivided and contains a tetraster. Figure 8 shows the same condition of the  $S_1$  cell and in addition we note the enucleated protoplasmic ball lying on top of it. Eggs showing tetrasters usually, if not always, possess this protoplasmic ball. In figure 7 it is present, but lies under the other cells and is not shown in the drawing. The amount of chromatin between the four centrosomes of the  $S_1$  cell is very large. It may be undergoing diminution or we may find the elongated chromosomes still present. In the latter case, one may usually count eight chromosomes lying in the spindles. In text figure D, an egg is shown with a history similar to that of the tetraster eggs, but for some reason, the centrosomes have failed to divide. In this respect it is exceptional, but eight chromosomes and the characteristic protoplasmic ball are both clearly seen.

The 2-cell stages usually show the  $S_1$  and the  $P_1$  blastomeres undergoing division (fig. 9). Except for the axes of division, which frequently do not occupy normal planes with regard to each other, the division figures are typical of the untreated eggs; and the lumpy condition of the chromatin of the  $S_1$  cell, which precedes diminution, is seen.

A number of smears of eggs were preserved when the embryos were in or just beyond the 4-cell stage, and in these, we find a

tremendous variation in the relative proportions in which the various abnormalities described above occur. In one preparation over 50 per cent of the eggs showed a tetraster in the  $S_1$  blastomere, or showed the later effects of it. In other slides only a very small proportion of the eggs showed this abnormality. The proportion of the eggs which showed an unequal distribution of the chromatin between the A and B blastomeres, showed the same variation in different slides. On the other hand, the failure of the blastomeres to form the rhombus is an abnormality found in a large per cent of cases on every slide.



D

Text figure D.

In the large amount of material which was preserved at frequent intervals after the 4-cell period, it has been possible to follow the results of the abnormal conditions described through the later development. Of course, as cleavage progresses, it becomes increasingly difficult to follow the course of the individual blastomeres, except in the case of the primordial germ cell. The latter is usually conspicuous on account of the large size of the nucleus. An analysis of the later stages has been facilitated by the use of clay models of the eggs and a comparison of these with the excellent figures given by Boveri ('99). In this way it has been possible to determine very exactly the plane in which a given cell is dividing, and the relation it will have to the rest of the blastomeres.

For the sake of clearness, the course of the different types of eggs will be followed separately through later cleavage in the following description. These will be taken up in the following order: (a) The effects of the abnormal positions which the  $S_1$  derivatives take in cleavage. (b) The result of the unequal distribution of the chromatin between the A and B blastomeres. (c) The fate of the tetraster eggs.

Among the treated eggs there is always a certain per cent which are perfectly normal. The 4-cell stage, such as shown in figure 5, is followed by the division of the A and B blastomeres in a plane approximately at right angles to the plane of the paper upon which the drawing is given (compare with fig. G). Following this, the  $P_2$  and EMSt cells divide in the median plane of the embryo (compare with fig. 1), and throughout the later cleavage, the analyses with models show that the normal development is continued.

The development of embryos in which the A and B blastomeres occupy abnormal positions in the 4-cell stage, may be followed with ease up to the time when the  $S_1$  derivatives number 16 cells. From this point on, such eggs are not to be distinguished from normal embryos. Figure 4 shows a typical case of the positions which the A and B cell take. In figure 10, we see these two cells dividing. The diminution process is taking place normally, but the planes which the dividing cells occupy, instead of being parallel (compare with the normal as shown in fig. G) are at right angles to each other. The result of such a division is shown in figure 11. In the egg shown in figure 12, the ectodermal cells are eight in number and the EMSt cell has divided in the median plane of the embryo. The  $P_2$  cell is in the equatorial plate phase of division. The elongated chromosomes characteristic of the primordial germ cell are clearly seen. In figure 13, we see a somewhat later stage. Both the  $P_2$  and EMSt blastomeres have divided. It is especially to be noted that the MSt blastomere does not lie in the median plane of the embryo (the EMSt cell divides into an E and an MSt cell; these normally lie in the median plane of the embryo, compare with fig. 1). It is very rare that we find the EMSt cell dividing in any plane but the

median, but in eggs examined *after* the division, we frequently find the MSt blastomere lying outside of the median plane. In these cases, the A and B derivatives are very asymmetrically distributed over the dorsal portion of the embryo and it seems that these cells push the MSt blastomere out of its normal position. This is more apparent on models of the eggs, of course, than in figures. The probable significance of this will be taken up later.

Many hundreds of eggs similar to those shown in figures 10 to 13 have been analyzed. The abnormal positions of the A and B cells are retained, there is no shifting to form the rhombus, at least it is not usually realized, the ectodermal cells derived from the S<sub>1</sub> blastomere take up positions on almost any part of the egg. Thus the bilateral symmetry of the embryo may be completely lost and, what is for our study more significant, the members of the ventral family, especially the MSt blastomeres, may be moved out of the median plane.

The most striking results of the unequal distribution of the chromatin between the A and B blastomeres is shown in figure 14. Aside from the positions which these two cells have, we see that one is dividing early and that it contains only a small number of somatic chromosomes in the spindle. The mate, on the other hand, shows no sign of division, and it will be noted that it contains a very large nucleus (compare fig. 14 with fig. 6). This result always follows the unequal distribution of the chromatin, apparently, and the early division of the one cell upsets the cleavage rhythm of the S<sub>1</sub> derivatives. If the distribution has been very uneven, then one of the cells may divide twice before its mate cleaves. With a more equal distribution the rhythm is not so markedly upset, but in any event the end result is the same: the S<sub>1</sub> derivatives become scattered irregularly over the surface of the embryo, the symmetry or balance of the embryo is upset, and probably members of the ventral family are pushed out of their normal positions, as was the case with the egg shown in figure 13.

The development of the embryos which showed a tetraster in the S<sub>1</sub> blastomere, is extremely variable, both in the number of cells formed by the division and in the distribution of the

chromatin. A typical case is shown in figure 15. Here there are six cells besides the  $P_2$  and EMSt blastomeres. One of these is evidently an enucleated protoplasmic ball, so characteristic of the tetraster eggs. The remainder came from the division. It will be noted that one of the small blastomeres contains a large amount of waste chromatin. This is a very common phenomenon exhibited by such eggs after division. In this egg, we also see that the MSt blastomere is not dividing in the same plane as the  $P_2$ . Such a condition is seldom met with.

The later development of these eggs which have had a tetraster in the  $S_1$  blastomere, is extremely abnormal. The  $S_1$  derivatives divide irregularly; they become scattered over the surface of the embryo or lie in one heap; and there is every indication that they very rarely or never form a cleavage cavity and gastrulate. In later cleavage stages, the abnormalities caused by these tetraster eggs is very striking. One of the marks of such eggs is the presence of the small cell with the large amount of chromatin (fig. 15). This disorganization, however, does not extend to the primordial germ cell, for we find it dividing normally in later stages when the embryo is otherwise totally abnormal.

At the time of gastrulation, a majority of the embryos appear normal or exhibit minor irregularities, such as a slight asymmetry of shape. The cleavage cavity is present in such eggs, however, and there seems to be no reason why they should not gastrulate normally. Among such embryos one finds a large number which have no cleavage cavity. This sometimes appears to be due to the fact that the ectodermal cells are too scattered or are too few in number to form it. But in every case the primordial germ cell nuclei may be clearly seen.

To sum up the foregoing description, we find that the abnormal eggs are of three types: (a) Eggs in which the A and B blastomeres have abnormal positions in the 4-cell and later stages. (b) Eggs in which there has been an asymmetrical distribution of the chromatin between the A and B cells. (c) Eggs with a tetraster in the  $S_1$  blastomere. We have now to inquire how these three abnormal conditions arose from the eggs just removed from the  $\text{CO}_2$ . And, secondly, what relation these ab-

normalities in cleavage bear to the embryos which had been allowed to undergo full development.

Taking up the first question, a close examination of the material has shown that the tetraster condition of the  $S_1$  blastomere and the irregular distribution of the chromatin in the A and B cells, is due to the same cause, that is, the fusion of the chromatin in the  $S_1$  cell (figs. 2a to 2d). A glance at these figures will show that in part of the eggs, only the ends of the chromosomes were involved and that the middle portions were free. In such eggs a division of the  $S_1$  blastomeres occurs but, owing to the fused condition of the chromatin, an equal distribution of it can not take place. Diminution of the chromatin occurs and one blastomere receives a number of small 'diminished' or somatic chromosomes, while the other cell receives, in addition to the somatic chromosomes, the whole mass of fused chromatin of the equatorial plate. If the fusion involved part of the chromatin which would normally go to form somatic chromosomes, then one cell would receive this together with the waste chromatin. When this fused mass goes to one cell, it does not undergo degenerative changes but (probably because of the presence of some somatic chromatin) it becomes resolved into a reticulum and fuses with the normal nucleus of the cell. In this way, one cell comes to contain more chromatin than its mate, and in later stages, the cell with the least chromatin divides earlier. Various stages of this process have been observed in my material.

When, however, the fusion involved all of the chromatin, as in figures 2b or 2c, then division appears not to take place. Apparently, the fused condition of the chromatin is responsible for this, but whether this prevented the centrosomes from going apart, or whether the fused mass kept the cell wall from cutting through, is not known. Stages that would decide this point have not been seen, but various other steps in the process have been observed. Thus the one cell comes to contain all the chromatin which should be distributed between blastomeres A and B. At the next division cycle, such eggs showed a tetraster in the  $S_1$  cell and eight chromosomes are found in the spindles (fig. D).

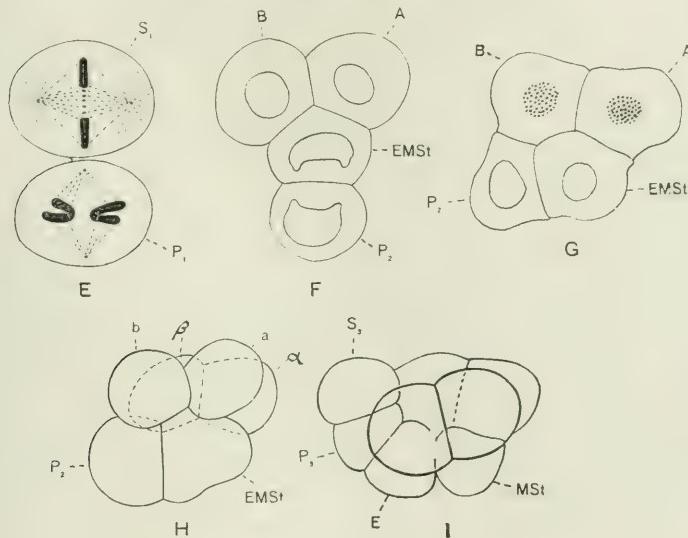
One very constant feature of the eggs showing a tetraster is the occurrence of a protoplasmic ball which lies on the blastomere showing this condition. There seems to be an intimate connection between the formation of the ball and the failure of the egg to divide. This point will be touched upon again.

The abnormal positions which the A and B blastomeres take up is to be traced, in part at least, back to the 3-cell stage, when the A and B cells separated (fig. 1). It is probable, however, that this is not the only source of the abnormality, for not infrequently, one finds eggs like those shown in text figure N. As may be seen in the figure, the A and B cell lie pressed against each other, and are dividing. The interesting thing is that the P<sub>2</sub> cell, is dividing in an abnormal plane. This condition could come about only in one of two ways. Either there has been a rolling or shifting of some of the cells, or, their polarity has been changed. Of the two possibilities the former seems the most probable since in the normal egg, a shifting process is involved which brings the B and P<sub>2</sub> blastomeres together (compare with figs. F and G). As we know nothing definite about the cause of the shifting in the normal egg, it is useless to speculate over the matter at this time. It seems worth while, however, to point out that the separation of the A and B cell might come about by an increase of surface tension. These cells normally lie pressed against each other, but were the surface tension of them increased, as through some action of the CO<sub>2</sub>, then they would separate. If the P<sub>1</sub> cell were also affected, the effect (that is, the separation) might be still more marked.

Before we take up the relation between the abnormalities found in cleavage and those exhibited by the fully developed worms, it will be necessary to describe a few of the more striking points in *Ascaris* development. Thanks to the works of Boveri ('99) and Zur Strassen ('96), we know the origin and fate of practically every cell in the young worm.

A few schematic sketches of the normal development are given in figures E to I. The first division results in two blastomeres, S<sub>1</sub> and P<sub>1</sub> respectively, following Boveri's nomenclature. These two cells have different potentialities. In the next division

cycle, these two cells divide in planes at right angles to each other (fig. E), and furthermore, as is well known, the chromatin of the  $S_1$  cell undergoes a process of "diminution" while the  $P_1$  cell retains the elongated chromosomes. The result of the division is four blastomeres which form a T-like figure (fig. F.). Following this, the cell marked  $P_2$  shifts around until it comes in contact with the B blastomere, forming in this way a rhombus. Up to this time, one can not speak of an anterior and posterior end of the embryo, but after the shifting, these parts are marked out. The



Text figures E to I

anterior end lies to the right in the figure, that is, at the A and EMSt side, while the posterior end is indicated by the  $P_2$  blastomere. The A and B blastomeres lie dorsally, as in the figure, the  $P_2$  and EMSt ventrally, the median plane of the embryo being parallel to the paper and passing through all four blastomeres.

The A and B cells now divide in a plane approximately at right angles to the median plane (fig. G), while the  $P_2$  and EMSt cells divide in the median plane (fig. I). The A and B cells give rise to the ectoderm covering the dorsal and anterior end of the body. The EMSt cell will give rise to the entoderm, part of the

mesoderm, and the cells of the stomodaeum. The  $P_2$  cell, after giving off several generations of ectodermal and mesodermal cells, forms the primordial germ cells. The most important points for us to remember are the following: During later development the A and B cells grow over the dorsal and anterior end of the body of the embryo. The EMSt and  $P_2$  cells lie ventrally and posteriorly and form the most important organs of the body. Nearly all of the posterior part of the body of the young worm comes from the  $P_1$  derivatives.

We can now turn to the question of the relation of the normal cleavage of the treated eggs and the worms which resulted from them.

Since a certain percentage of eggs always developed in a perfectly normal fashion during cleavage, it is clear that the fully developed normal worms, such as shown in figure A, arose from this source.

It is equally clear that the masses of totally disorganized cells which one finds in figure C are due, in part at least, to the formation of the tetraster in the  $S_1$  blastomere, with the subsequent abnormal development. No doubt other sources contributed to this class of embryo.

The embryos in which the posterior end is only partially differentiated are undoubtedly to be traced to the eggs with the A and B cells lying in abnormal positions, but the details of how this condition affected the later development are uncertain because we have so little knowledge in how far the later shifting of the blastomeres in *Ascaris* is due to internal organization, and in how far to simple mechanical relations, such as mutual pressure, etc. Admitting this uncertainty at the start, we may give a very simple explanation which appears to agree with all of the observations recorded.

A glance at figure I will show that the derivatives of the  $P_1$  cell form a sort of half keel on the ventral and posterior end of the embryo, and the ectodermal cells, (derivatives of the  $S_1$ ) by their division form a more or less symmetrical covering for this. The works of Boveri ('09) and Miss Stevens ('09) have shown that this ventral keel may take place when the A and B derivatives

are absent (as when the S<sub>1</sub> is killed by ultra-violet light). This indicates a high degree of internal organization in these cells. Here it may be remarked that in these experiments there were no ectodermal cells which might move the members of the ventral family out of the median plane. Were, however, the A and B derivatives present but in positions which would destroy the symmetry of the embryo (for example, were they lying only on one side of this keel, and I have observed cases which approached this) to unbalance the system, so to speak, then it seems very probable that some of the ventral cells would be moved out of the median plane.

In the later development of the eggs in which the A and B cells occupy abnormal positions, we seem to see this unbalancing taking place. Quite frequently in such eggs, the majority of the A and B derivatives lie on one side of the keel, and in the later stages, as shown in figure 13, one of the blastomeres (the MSt in this case) is moved out of the median plane. Prior to the division, the EMSt blastomere lies in the median plane and even in the metaphase, it holds this position. I have noted only one or two exceptions to this. After division, however, the MSt cell is frequently found lying out of the median plane, and the most probable explanation is that it has been moved out of this plane by the overlying ectodermal cells. It may well be that other causes were operating to produce the same end effect. In a few cases, the EMSt blastomere divided in an abnormal plane. figure N shows such a case, or, disorganization may have come later.

As cleavage went forward in these eggs, the A and B derivatives formed the cleavage cavity by mutual pressure and gastrulation took place. It was not until organ formation began that the effects of the shifting of the MSt cell could be observed. Looked at from a theoretical point of view, since the MSt blastomere forms the cells of the stomodaeum (after several divisions) we should expect that were this cell pushed out of its normal position, the resulting embryo would lack this part. The embryos in which the anterior end is disorganized but in which the posterior end is normal, seem to fulfill these expectations. The

posterior end, coming from the other end of the keel and more or less independent of the derivatives of the  $S_1$  cell, would be normally formed, since all the necessary elements were present. It is not to be expected that simply the MSt cell would be affected. No doubt the failure of this cell to take up the proper position causes the whole anterior end to be disorganized, and when the A and B derivatives were very irregularly distributed (when they, for example, lay on top of the  $P_2$  cell without touching the EMSt, and I have observed such cases) the disorganization probably extended to the posterior end. It seems likely that the degree to which the posterior end was differentiated is to be correlated with the positions which the A and B derivatives took, and thus we have a series of stages from worms which are seven-eighths normal, to worms in which only the stump of the posterior end is differentiated.

In this way we are able to explain the production of the half embryos following the treatment of the eggs with CO<sub>2</sub>. There is, of course, another way of explaining their production, but this has not been advanced because it did not harmonize, as it seemed to me, with the facts which have been discovered by Boveri and his students. We have assumed that the ectodermal cells coming from the  $S_1$  blastomere, were more or less indifferent in their nature. This is indicated by the normal development, for the cells divide rhythmically and form the general ectodermal covering for the anterior end of the body. In contrast to these, the cells of the ventral family ( $P_1$  derivatives) divide very irregularly and possess a high degree of specificity, that is, one forms the entoderm, another the mesoderm, or primodial germ cells, and so on. Were we to attribute specificity to the ectodermal cells, then the explanation for the half embryos would be that they were formed since the cells destined for the anterior end were scattered and this part of the embryo was undifferentiated.

The latter view does not seem tenable since all work points to the indifferent nature of the  $S_1$  derivatives. In any event, however, the production of the half embryos is to be traced to the abnormal positions which the A and B blastomeres take in the 4-cell stage.

While the great majority of the eggs followed the different types of development outlined above, a small per cent could usually be found in any slide which were abnormal for no apparent reason. One condition rather common, is that seen in figure N, where we find the A and B cells undivided even after the P<sub>2</sub> and EMSt cell have nearly completed their division. What the fate of such eggs is can not be definitely stated, but since we may find the A and B cells undivided in later stages, it seems probable that such eggs never gastrulate. The percentage of eggs of this type is small, in any event.

We now come to the question, why are some of the eggs affected by the treatment with the CO<sub>2</sub>, and why do others develop normally under the same conditions? And why do we find different proportions of abnormalities in the smears of the same female? It was for the solution of these questions that two series of experiments were planned and attempted, but since these were unsuccessful, the working hypothesis upon which they were based, will be given. This explanation is only tentative.

It is well known that in any mass of *Ascaris* eggs, some develop more rapidly than others. When the eggs were placed in the CO<sub>2</sub> they underwent a certain amount of development before the oxygen available was exhausted. When the supply of oxygen lasted until the nuclei were in the resting stage, no ill effects resulted except such as might arise from a shifting of the blastomeres. If, however, the eggs were in the equatorial plate phase when the supply of oxygen gave out, then they remained in this state until brought into the air again. Whether the fusion of the chromatin took place in the CO<sub>2</sub> or whether it resulted later, was to have been determined.

The variation in the proportion of the abnormal types is, without doubt, due to the following reason. All the eggs for the present study were taken from the fresh uterus of a single female. Such eggs removed from the end of the uterus have given off their polar bodies and the male and female pronuclei lie side by side until oxygen is admitted. Eggs lying farther back in the uterus are not so far advanced as those lying at the tip, con-

sequently, some of the smears are advanced farther than others; a larger proportion of eggs reach the equatorial plate phase in one slide than in another, and we find as a result a larger proportion of tetrasters. It seems probable that in the smear with over 50 per cent of the eggs showing tetrasters in the  $S_1$  cell, the  $S_1$  blastomere had been able to bring its division only to the equatorial plate phase. However, on all these points more experimental evidence is needed and the author hopes to fill in the gaps as soon as suitable material is available.

It is of further interest to ask how the eggs were able to live and develop to a slight degree, in an atmosphere of  $\text{CO}_2$ . Especially, when they had been kept at a temperature where they would normally have developed in some three weeks. These questions are taken up in the following discussion.

It has long been recognized that intestinal parasites live under anaerobic conditions, but Weinland ('01) was the first to show the mechanism by which they obtained the oxygen necessary for their existence. It had been known previously that *Ascaris* contained a large amount of glycogen, and Weinland was able to show that when these animals were kept in a medium without food, this glycogen disappeared and he obtained  $\text{CO}_2$  and valeric acid. He suggested that the glycogen had been broken down by some animal ferment. Glycogen is one of the complex sugars with the empirical formula of  $(\text{C}_6\text{H}_{10}\text{O}_5)^x$ . According to Weinland's ideas, this is broken down in essentially the following way:



A number of authors (Brault and Loepers '04; Buschs '05, '06; Kemnitz '11; and Brammertz '13) have shown that the eggs of *Ascaris* contain large amounts of glycogen. Brammertz was able to show that during the formation of the polar bodies, the amount of glycogen diminished in the region where these bodies were being formed. The reason for this was that the glycogen was broken down to furnish the oxygen necessary for this process. For further development the oxygen of the air seems to be essential, although the glycogen in the egg is

slowly used up as development goes forward. Brammertz regards this glycogen as a sort of reserve to tide the embryo over unfavorable conditions. He cites one experiment in favor of this view, which is quite similar, in its conditions, to the experiments recorded above. He found that if eggs were placed in 70 per cent alcohol, part of them developed as far as the two cell stage before they were penetrated by the alcohol and killed. He regards it as improbable that the eggs could have gotten the oxygen necessary from the alcohol, and thinks there is proof here that the glycogen was used for this purpose.

The author has not made any experiments with the eggs treated with CO<sub>2</sub> but the conditions are so similar with the experiment cited that it seems very probable that the eggs used in my experiments were able to live over this period of three months because of the presence of the glycogen stored in their protoplasm.

#### ANOMALIES

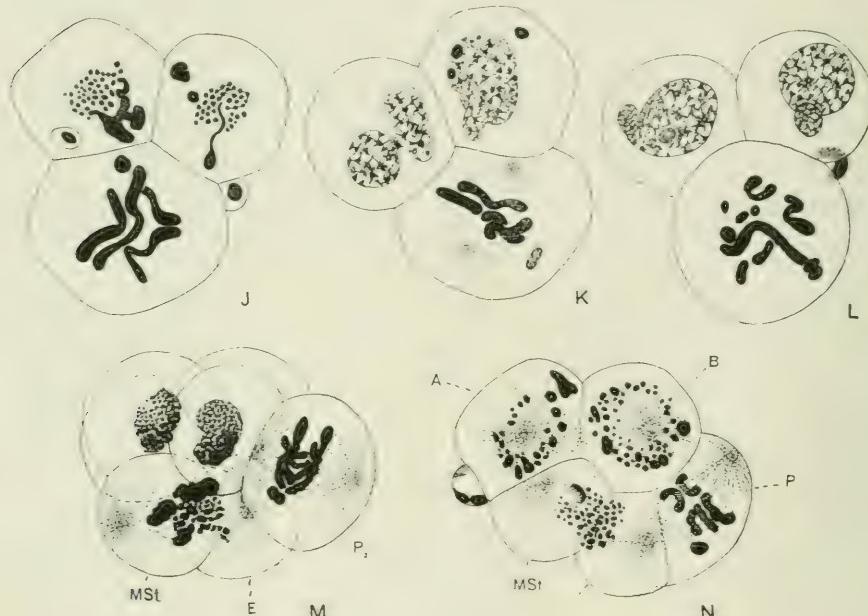
Under this heading I wish to record some observations made on the treated eggs, which have a bearing on the problems of sex determination and the cause of diminution in *Ascaris megalcephala*.

In the dividing primordial germ cells, occasionally eggs have been seen in which there were, besides the four chromosomes, additional elements (in 13 cases out of 123 eggs taken at random). Typically there is only one additional chromosome, as in figure J, but cases with two, three, four, and even eleven fragments have been seen (fig. L). The way in which the single element behaves during division is shown in figures K, M, and N. Cases with more fragments could not be followed through division.

The presence of one or more chromosome fragments in the primordial germ cells of *Ascaris megalocephala* have been described by a number of authors, and at the present time two views have been advanced to explain them. According to Boring ('09) and Boveri ('09), they represent the accessory chromosomes in this species. This is the view generally accepted. Kautsch ('13), however, has shown that another interpretation

is possible. After an extensive study of anomalies in *Ascaris*, he comes to the conclusion that these fragments are probably bits of chromatin brought into the egg when the polar bodies did not receive their full share of chromatin.

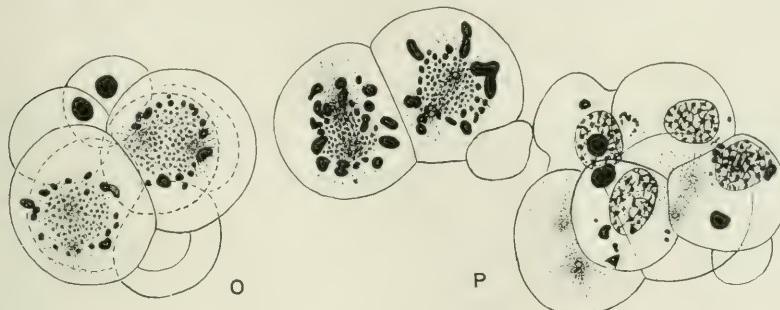
Kautsch approached the question of the accessory chromosome in *Ascaris* in another way. He counted the number of somatic chromosomes in eggs in which there was only one chromosome,



Text figures J to N

and he found that they fell into two numerical groups; one centering around 27, the other around 36, somatic chromosomes. These, he suggests, may be male and female numbers. The counts made by Kautsch are too few in number to be conclusive, but it is interesting to note that his view falls into line with the work done by Edwards ('10) on *Ascaris lumbricoides*. The latter author, as is well known, found that the accessory chromosome in this species, is represented by a group of five small chromosomes.

The observations recorded above, have a certain bearing on the question, since it is clear from them that genuine fragmentation may take place in the chromosomes of *Ascaris megalcephala*, in some cases all four chromosomes being affected. The fragmentation of the chromatin in figure L, however, certainly has nothing to do with the accessory chromosome, and since a series may be formed in which one, two, or even all four chromosomes may be broken up, it becomes a question whether or not we can interpret such cases as shown in figures K, M, or N, as having any relation to the accessory chromosome. Just what relation the fragmentation in the normal eggs has to do with that observed in the eggs treated with CO<sub>2</sub>, is uncertain, but it does not



Text figures O and P

seem improbable that they are both expressions of a tendency residing in the chromosomes.

A second anomaly found is shown in figures O and P. In figure O, the P<sub>2</sub> and EMSt cells are both dividing, and it will be seen that both are undergoing diminution. It is especially to be noted that the spindles of the two cells are not parallel. In figure P, another egg is shown in which both the P<sub>2</sub> and EMSt cells are undergoing diminution. A detailed drawing of the two cells is seen to one side. It is especially to be noted that a protoplasmic ball lies on one of the two cells.

In figure 16 is shown a rare case where the P<sub>2</sub> and EMSt cells are fusing after division. Note particularly the protoplasmic ball coming from the fusing cells. (The S<sub>1</sub> cell is seen lying be-

neath; it is clearly undergoing diminution.) In figure 17 is a later stage in which the fused  $P_2$  and EMSt cell is undergoing division. It will be seen from the figure that there are four small cells present which contain waste chromatin, besides two protoplasmic balls without any chromatin. The presence of a large amount of chromatin in one small cell indicates that the four small cells have arisen by the division of a tetraster. Compare this egg with that shown in figure 15. The presence of the protoplasmic balls indicates the same thing. One lies on the fused  $P_2$  and EMSt cells, and has arisen, probably during division, as in figure 16. The other protoplasmic ball was undoubtedly formed when the  $S_1$  cell failed to divide. The interesting thing about this egg is, that the cell which I interpret as coming from the fusion of the  $P_2$  and EMSt blastomeres, such as we see in figure 16 is undergoing division; shows a tetraster and as may be seen in the figure, the chromatin is undergoing diminution. Only one case of this sort has been seen, but it has been very carefully studied and there can be little doubt of the correctness of the interpretation given.

I do not propose to take up a detailed discussion of the question, "What causes the diminution in the somatic cells of *Ascaris*?" And yet the question can not be fully omitted since the observations recorded in the foregoing pages throw some light on the subject, even though they do not give a final answer. It is well known that two views have been held with regard to this subject. Zur Strassen ('06) invoked what was essentially a qualitative division of the chromosomes, in order to explain the phenomenon. This view has been contested by Boveri ('10), who, by his masterly analysis of dispermic and centrifuged eggs in *Ascaris*, showed that the explanation advanced by Zur Strassen was untenable. Finding it impossible to explain the cause of the diminution to factors residing in the chromosomes themselves, Boveri turned to the cytoplasm and advanced his 'Schichtung' hypothesis. This author, convinced of the heterotrophic nature of the protoplasm in *Ascaris*, conceives of the various substances being arranged in layers. The blastomeres receiving certain layers of protoplasm, undergo diminution while

other cells not receiving these layers retain the elongated form of the chromosomes. A considerable body of evidence has been produced by Boveri to substantiate this view.

From time to time, various authors have noted exceptions to the rule that the germinal cells never undergo diminution. Most recently we have the work of Kautsch who has given drawings of a number of cases and points out that in all of the eggs observed by him, the axes of the spindles in the P<sub>2</sub> and EMSt cells were parallel. This the author took as indicating that the protoplasm of the two cells was similarly structured. How the condition arose, he does not state. A glance at either figure O or P will show exceptions to this rule. In fact, in the number of eggs in which I have observed this anomaly, I have been unable to find any two spindles which were parallel.

Viewed in the light of Boveri's hypothesis, the two eggs shown in figures 16 and 17, prove very interesting. Here we have the P<sub>2</sub> and EMSt blastomeres fused together after division, and, if the diminution process depends on a qualitative division of the chromosomes, we should find some somatic and germinal chromosomes in the spindles. But as will be seen from figure 17, this is not the case. On the other hand, if the presence of the elongated chromosomes depends on some specific substance carried in the cytoplasm of the primordial germ cell, then we should expect to find eight complete chromosomes in the spindles.

A glance at figure 17, shows that the chromosomes are undergoing typical diminution. How is this to be explained by the hypothesis advanced by Boveri? Can it be that the germ path determiner (or however we choose to think of this postulated substance) has lost its potency over the protoplasm which it had controlled in the preceding division? Or, is the explanation for the diminution process to be sought in some other theory? The answer to this question is, I think, given by the protoplasmic ball which almost invariably is found in eggs showing diminution in the germinal cells.

If we conceive of the presence of the elongated chromosomes in the germinal cells as being due to some finely balanced qualitative or quantitative chemical inter-reactions, then it is easy

to imagine that any process which would remove part of the protoplasm or otherwise disturb the relations, would upset the reaction. Or, if we think of some specific substance, a true germ path determiner, as being present in the germinal cells, the loss of part or all of this substance would produce diminution in such cells. Which of these two views will prove the correct one, it is impossible at the present time to say, but the evidence to be presented can be equally well interpreted for either case.

In the eggs treated with CO<sub>2</sub> the quantitative relations are frequently upset by the formation of protoplasmic balls. These balls are apparently formed by the giving away of the cell wall when the cleavage pressure is at its height. It is invariably found when the A and B blastomeres fail to separate. More rarely, it may be given off from the dividing primordial germ cell. In figure P we have such a case, associated with the diminution process in the germinal cells. Most of the eggs which have shown diminution in the germinal cells, have been characterized by the presence of such a ball. In glancing over the figures given by Kautsch, I notice also the frequent appearance of such a ball, although Kautsch does not mention them in his description of these abnormal eggs. Finally, in the eggs shown in figures 16 and 17, we see a large mass of protoplasm has been cut off from the germinal cell. The constant occurrence of the protoplasmic ball with the diminution going on in the primordial germ cell has convinced me that the two have a close relation. Here we seem to have an explanation for the diminution process, for, when this mass of protoplasm is thrown out of the germinal cell, substances (such as the germ path determiner itself or some material necessary for its action) are either removed, or the balance between the inter-reacting chemical substances is upset. Either of these causes would probably be sufficient to bring about the diminution. In the case of the ectodermal cells, A and B, the formation of the protoplasmic ball has no effect on diminution since the substance inhibiting this process is not present.

In advancing this explanation, it is not my intention to imply that diminution in the germinal cells takes place only after the formation of the protoplasmic balls. In fact, I have myself found cases in which diminution was plainly taking place, but I was unable to find any ball. Such eggs, however, were invariably abnormally retarded in their development and the explanation for the diminution is probably to be found in another cause. The author ('15) has shown that in the sea urchin egg, after fertilization, progressive changes are going on in the cytoplasm of the egg which are independent of the cleavage process and thus we may have the formation of the micromere, which normally comes in the 16-cell stage, in the 8- or 4-cell stage. It is very probable that this process is characteristic of all eggs. Thus in the germinal cells of Ascaris we may imagine series of changes are going on, only here they tend toward different results. Were the germinal cell long delayed in its division it may be that the somatic tendency becomes so strong as to suppress the germinal one, and thus diminution takes place.

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## PLATE 1

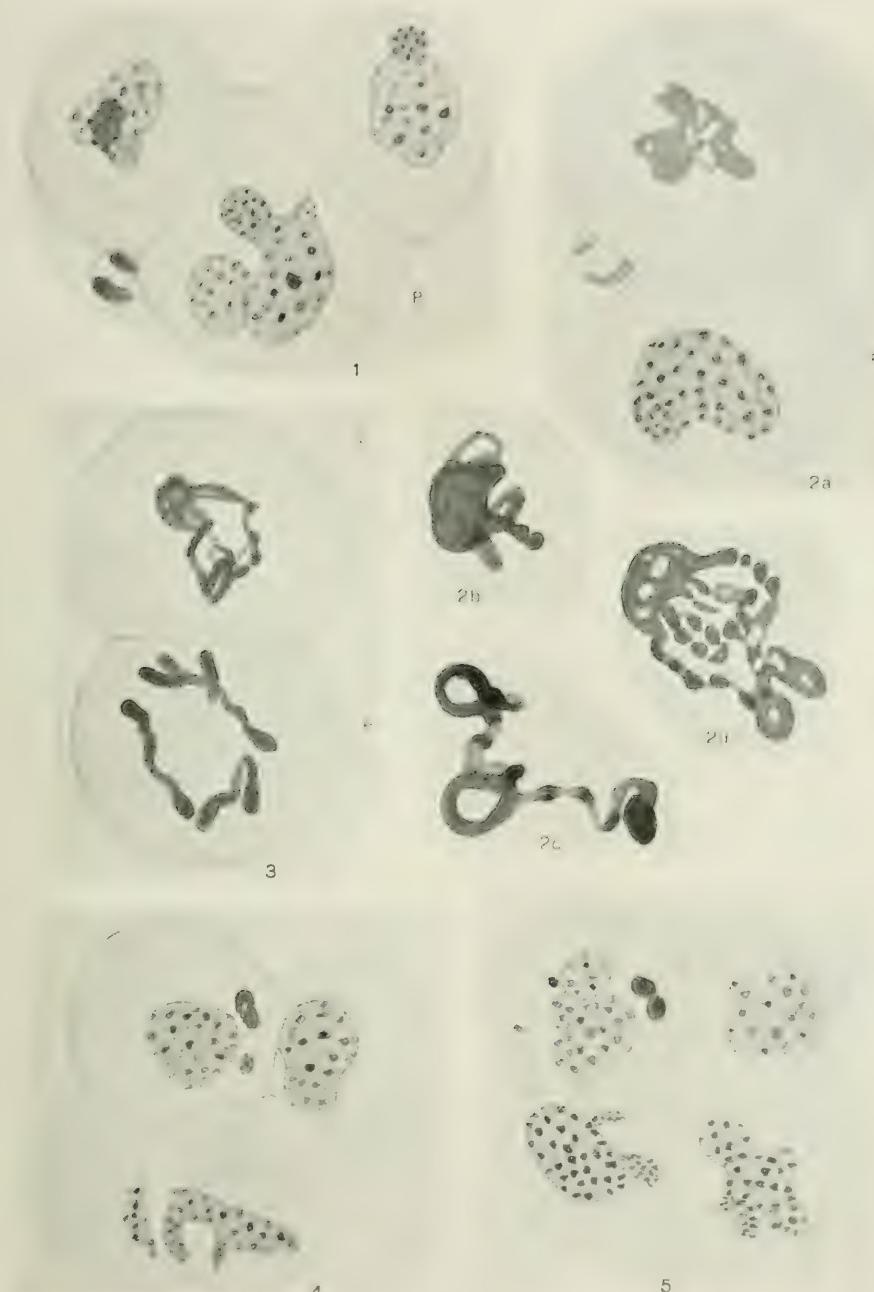
## EXPLANATION OF FIGURES

All drawings made with camera lucida, and  $\frac{1}{2}$  oil immersion. Figures 2b, 2c and 2d are drawn at a higher magnification than the remainder of the figures.

- 1 A 3-cell stage showing the separation of the A and B blastomeres.
- 2a A 2-cell stage showing the peculiar fusing which the chromatin in the S<sub>1</sub> blastomeres.
- 2b, 2c, 2d Detailed drawings of the fused chromatin.
- 4 Showing a 4-cell stage with the A and B cells occupying abnormal positions.
- 5 A 4-cell stage, normal after treatment with CO<sub>2</sub>.

EFFECTS OF CARBON DIOXIDE ON EGGS  
THEOPHILUS S. PAINTER

PLATE I



## PLATE 2

### EXPLANATION OF FIGURES

- 6 A 3-cell stage showing the unequal distribution of the chromatin between the A and B cells.  
7 Showing the tetraster in the S<sub>1</sub> blastomere.  
8 Showing the tetraster in the S<sub>1</sub> blastomere.  
9 Showing normal 2-cell division after treatment with CO<sub>2</sub>.  
10 Showing the A and B blastomeres dividing in abnormal planes.  
11 Showing the result of the division when the A and B cell occupy abnormal positions.

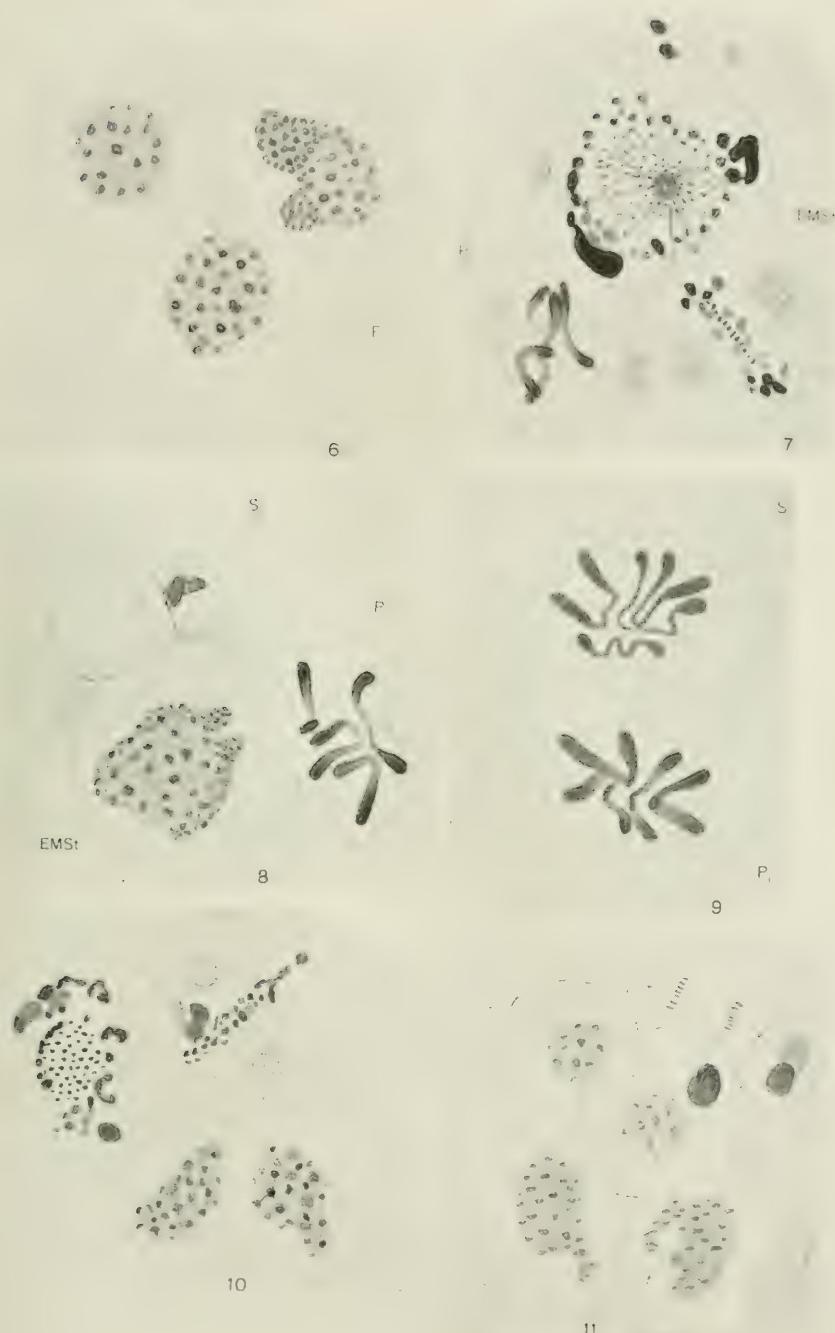


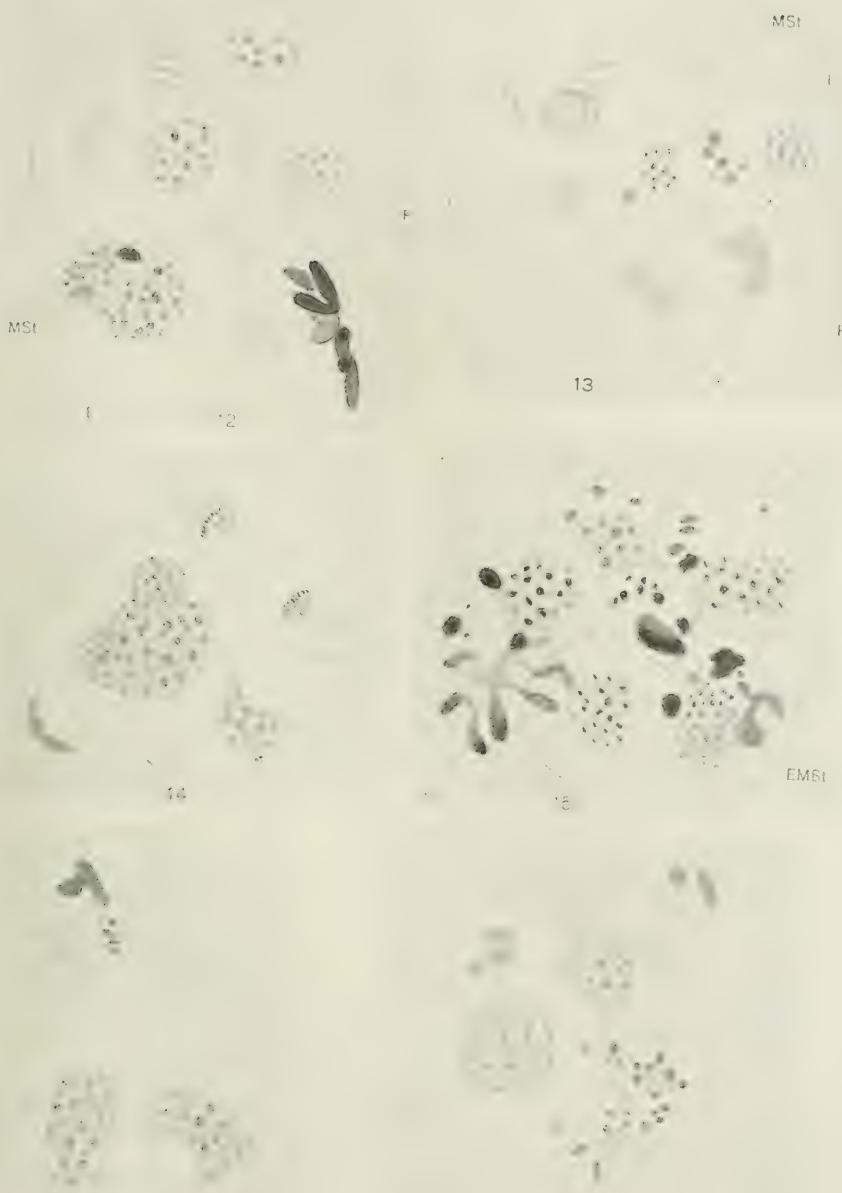
PLATE 3

EXPLANATION OF FIGURES

- 12 Showing the effect when the A and B cell divide in abnormal planes; the ectodermal cells number 8 in this egg.
- 13 A slightly later stage of the same; note that the MSt cell does not lie in the median plane of the embryo.
- 14 An egg showing the effect of the unequal division of the chromatin between the A and B blastomeres.
- 15 An egg showing the effect of the tetraster formation in the S<sub>1</sub> cell.
- 16 Showing the P<sub>2</sub> and EMSt cell fusing; note particularly the protoplasmic ball projecting from the fusing cells.
- 17 Showing the later effect of the fusion of the P<sub>2</sub> and EMSt cells; note that diminution is taking place in this cell.

EFFECTS OF CARBON DIOXIDE ON EGGS  
THEOPHILUS S. PAINTER

PLATE 3





771

# VARIATION AND INHERITANCE IN ABNORMALITIES OCCURRING AFTER CONJUGATION IN PARAMECIUM CAUDATUM

RUTH J. STOCKING

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## TWENTY FIGURES

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## I. INTRODUCTION

The fact that there are two kinds of teratological variation, one caused by gametic constitution and therefore heritable, the other occasioned by the action of environmental conditions, and not heritable, has been recognized for some years. There was for a time an endeavor on the part of many investigators to show that all abnormalities were due to conditions of nurture; but the discovery of the strict mendelian inheritance of a large number of malformations has overthrown that theory. In many of the metazoa both kinds of teratological variation, gametic and environmental, have been studied, and their causes and behavior partly determined. In the protozoa the abnormalities caused by the action of external factors have received by far the great-

<sup>1</sup> Part of the work here reported was completed while the author held the Alice Freeman Palmer Research Fellowship of Wellesley College.

est amount of attention, heritable abnormalities having been but little studied. This paper presents an investigation of heritable abnormalities in *Paramecium*.

The principal points examined in this presentation are the following: The origin and nature of the abnormalities; their relation to conjugation; their inheritance and variation in uniparental reproduction, with relation to the present day problems of 'pure line' work; how precisely inheritance occurs; whether there are variations of kind and degree of abnormality; whether such variations are themselves inherited; whether by selection abnormal stocks multiplying asexually can be altered in their hereditary characteristics or differentiated into two or more hereditarily diverse stocks; their relation to biparental inheritance; their relation to survival.

Previous work on the abnormalities found in protozoa has dealt mainly, as before remarked, with the results of environmental action. Jollos ('13) subjected a race of *Paramecium caudatum* to changes of temperature and obtained by this method differences in size, as did Hertwig ('08), Popoff ('08, '09), and Rautman ('09). Jollos found that these changes are transitory and soon disappear as the paramecia become adapted to their new conditions. In a short preliminary note, which does not present the evidence for his conclusions, he states that in one case he obtained a permanent change. He subjected a race of *Paramecium* to a high temperature and obtained a race which was permanently small at high, normal, and low temperatures. Popoff ('09) was able to produce by experimental means two abnormal races of *Stentor*, one giant and the other dwarf. He centrifuged a dividing *Stentor*, and so caused an unequal distribution of the nuclear material. The daughter cell which received the smaller part was one-fourth the size of the cell which received the larger part. These two multiplied normally for about a week and during that time retained their abnormal sizes. The cultures were then lost. He obtained another giant race of *Stentor* by suddenly cooling a dividing individual. The division did not take place and the animal reorganized into a single individual which grew and subsequently

divided, forming a giant race which was kept for about forty-five days, and during that time bred true. Lewin ('10) obtained abnormally-nucleate races of *Paramecium* by cutting an individual through the macronucleus. The production of form abnormalities in *Paramecium* by cutting and other experimental means has been studied by Calkins ('11), Peebles ('12), Balbiani ('93), McClelland ('09), and Jennings ('08). They all found that such experimentally produced abnormalities are mechanically handed on to one daughter cell at each division for a longer or shorter time. But such abnormal forms are gradually remodelled during successive generations, or die, and their normal sisters show no tendency to produce abnormal progeny. The teratological variations that arise spontaneously in a culture multiplying vegetatively have been studied extensively by Jennings ('08). With one exception he found that all such forms either die very soon or give rise to a race of normals. In one case he did find that the abnormal individual gave rise to a race of abnormals, the deformity being such as to prevent the daughter cells from separating after division, thus forming what have been called 'double monsters.' In all other cases the abnormality was not a race character but an individual character and was not inherited. During the course of a year's work at the Johns Hopkins University I followed the history of several abnormal forms which had arisen in the cultures of normals being carried on by other workers in the laboratory. In no case did these forms give rise to a race of abnormals; in one case a race of normals resulted; in all other cases death occurred either before any divisions had taken place or after one or two irregular divisions. In rare cases, Jennings ('13) found, abnormalities may arise in the members of split pairs; that is, in the members of pairs separated before conjugation has been completed. But these also never gave rise to a race of abnormals, either dying very soon or becoming entirely normal.

Teratological variations arising soon after conjugation have been described in a few cases. Simpson ('01) observed four daughter cells of a normal exconjugant *Paramecium*, three of which were normal while the fourth was posteriorly split. This

animal divided eight times and its history was similar to that given above for the experimentally produced abnormalities in *Paramecium*. The abnormal character was handed on to one cell at each division, and the abnormal animal produced by the eighth division died. All of the sister cells and their progeny were normal. The only other observation known to me on the abnormalities arising after conjugation is made by Jennings in his 1913 paper quoted above. While he found only extremely rare and transitory abnormalities among his 'split pairs' and 'free' individuals, he found a large proportion of malformations among his exconjugants and their progeny. He describes their different types, characteristics, and constant and continued appearance throughout the course of his experiments; and adds, "A precise study is greatly needed, as to the minute characteristics of these abnormalities, their heritability, their experimental cause, and their cytological basis." It was at his suggestion that a series of experiments was started for the purpose of studying these problems, and under his direction that these experiments have been carried through. I wish to express here my most sincere thanks for the constant help he has given during the entire course of the work.

## II. METHODS

In all of the cultures on which this work is based the method of handling, cultivating, and recording was identical with that described by Jennings ('13). Conjugation was first induced; after the pairs had separated the two members were isolated and cultivated separately in  $\frac{1}{16}$  per cent Horlick's malted milk (Peebles, '12). Each pair was numbered, and the two members of a pair designated *a* and *b*; the races which arose from these exconjugants were called after them in the same way, *2a*, *2b*, *3a*, and so on. Beside the slide cultures, mass cultures were also kept for each race. These were kept in bottles and each bottle was labelled correspondingly, *2a*, *2b*, *3a*, and so on. The method of recording described by Jennings was supplemented in my work by drawings of the abnormal forms. In a few cases

this was impossible because of the rapid movements of the animal. For about two weeks after conjugation camera lucida drawings were made of the abnormal forms. These were often less active than the normal forms and after being in the same drop of culture fluid for two days all of the individuals were more or less sluggish. So with a little patience camera lucida drawings could be obtained of almost all the abnormals. When the number to be examined and drawn became very large, however, there was no time for that procedure and free hand drawings were made in the majority of cases, camera lucida only in such cases as seemed of more than ordinary interest. The slide cultures were examined every other day during the cool weather, every day when it became very warm, the individuals counted, the abnormals drawn, selection made of those by which the slide cultures should be carried on, and the others put into the bottle mass-culture corresponding to that race. The individuals selected for slide culture were then washed in the milk solution, and put into fresh fluid on clean slides.

### III. EXPERIMENTAL CULTURES

The material presented in this paper is derived from three experimental cultures carried from the fall of 1912 to the spring of 1914. The first experiment, conducted from December 2, 1912, to June 11, 1913, was carried out with exconjugant members of a wild population which doubtless included members of many diverse stocks. The chief aim of this first experiment was to ascertain whether or not abnormalities were ever inherited, and to what extent. From November 25, 1913 to December 22, 1913, a second experiment was conducted on a group of animals all descended from one individual, constituting therefore a 'pure line' or 'clone.' It was concerned mainly with determining the effect of conjugation among the members of a single clone, particularly as to the production of abnormalities; this was brought out by a comparison of exconjugants with members of split pairs belonging to the same clone. In the first experiment some work on the effect of selection was attempted; but the third experiment, carried out on a wild population from

January 14, 1914, to April 1, 1914, was concerned wholly with that problem. In it an attempt was made to ascertain the efficacy of selection as a means of breaking a single clone into two or more lines differing hereditarily in amount or kind of abnormality.

In all three of the experiments, in two of which very large numbers of exconjugants and their descendants were studied, a large proportion of the resulting races were abnormal, just as was the case in the exconjugants studied by Jennings ('13). In Experiment 1, with a wild population, the proportion of abnormalities was 36 per cent of the entire 262 exconjugants. In Experiment 2, with the members of a clone, the proportion of abnormalities was much higher, being 81 per cent of the whole 200. In Experiment 3 the number of exconjugants studied was small; the abnormal races formed 43 per cent of the total 28. In the 54 members of the 27 split pairs of Experiment 2, twenty of which were cultivated for 19 days, and thirty-four for 7 days, no abnormalities at all appeared. Whenever sets of individuals are isolated without conjugation and cultivated in the same way, no such proportion of abnormalities are observed; indeed as a rule no abnormalities whatever are obtained. We shall return later to the relation of the abnormalities to conjugation (page 412).

The exconjugants from large cultures of paramecia may be divided, on the basis of their subsequent history, into a number of diverse classes, which are summarized for our three cultures in table 1. 1) A few pairs, in some cultures, never separate, but die while united. 2) A considerable number, in some cases, die within 24 hours after separation. 3) Others live after separation—often for a long time—but never divide. 4) A fourth group divide, but produce individuals that are in some way and to some degree abnormal. 5) Finally, a certain proportion propagate normally after conjugation, giving rise to typical progeny. The proportions of these different classes are shown in table 1.

The single pair that did not separate lived united for five days. The exconjugants that never divide after separation form a large proportion of the abnormal individuals, rising to 28 per cent of

TABLE 1

*Diverse types of exconjugants, their number and proportion in the three experimental cultures*

EXPERIMENT	1) NEVER SEPARATED		2) DIED FIRST DAY		3) NEVER DIVIDED		4) PROGENY ABNORMAL		5) PROGENY NORMAL		TOTAL
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
	1	0	25	10.0	74	28.0	21	8.0	142	54.0	262
2	2	1.0	3	1.5	27	14.0	133	66.0	35	17.5	200
3	0	0	0	0	0	0	12	43.0	16	57.0	28
Total.	2	0.4	28	5.7	101	20.6	166	33.9	193	39.4	490

all in Experiment 1. These often live for some time, the length of life varying greatly. The length of life in days for the 101 exconjugants that never divided was as follows:

Length of life in days.....	1	3	5	7	9	11	13	15	17	Total
Number of exconjugants:										
Experiment 1.....	0	0	46	10	4	5	5	3	1	74
Experiment 2.....	2	8	4	2	1	10	0	0	0	27
Total.....	2	8	50	12	5	15	5	3	1	101

All of the animals of Experiment 2 which never divided were dead by the thirteenth day after conjugation; in Experiment 1 nine such individuals lived for some time after this. Those that live for a number of days often change a great deal in size and shape; many of them become immensely large. Twenty-eight of these individuals were measured; for their lengths and diameters on successive days after conjugation see p. 474.

The diversities of size and shape among these individuals is illustrated in figure 1, which shows nine of the individuals of Experiment 1 which never divided, all camera lucida drawings to the same scale. The usual size of *Paramecium caudatum* on the same scale is shown in figure 5.

The largest single individual *Paramecium* that I have ever seen was the first individual listed below for Experiment 1; it measured 520 by 150 microns, as against a usual length of 150 to 200 microns. The smallest individual that never divided was likewise in Experiment 1; it measured 128 by 10 microns. It is evident that there was much greater variability in size, shape,

and length of life, in these individuals of Experiment 1, than in those of Experiment 2. It appears probable that this is con-

*Experiment 1*

MEASUREMENTS ON

Seventh day	Ninth day	Eleventh day	Thirteenth day	Fifteenth day
520 x 150	340 x 130	320 x 100	220 x 55	
450 x 120		420 x 150		
420 x 92	260 x 80			
400 x 140		420 x 120		220 x 90
400 x 135	390 x 160			
350 x 110				
350 x 80				
340 x 110		350 x 130		
340 x 100				
340 x 70			220 x 105	
330 x 100		240 x 150		
300 x 120				
290 x 140			240 x 105	
290 x 100		300 x 170		
280 x 140				
210 x 115				
200 x 20		210 x 20		
190 x 160				
165 x 20	250 x 50			
129 x 10	200 x 35	250 x 60		
Ave. 315 x 102	288 x 91	314 x 112	227 x 88	220 x 90

*Experiment 2*

MEASUREMENTS ON

Third day	Fifth day	Seventh day
240 x 75		
240 x 55		
215 x 35		
210 x 65		
195 x 55	145 x 35	150 x 20
190 x 45		
175 x 35	145 x 35	
Ave. 209 x 52.....	145 x 35	150 x 20

nected with the fact that the former culture consisted of wild individuals, probably of diverse stocks, while the latter consisted of members of a single clone. This matter will be taken up later.

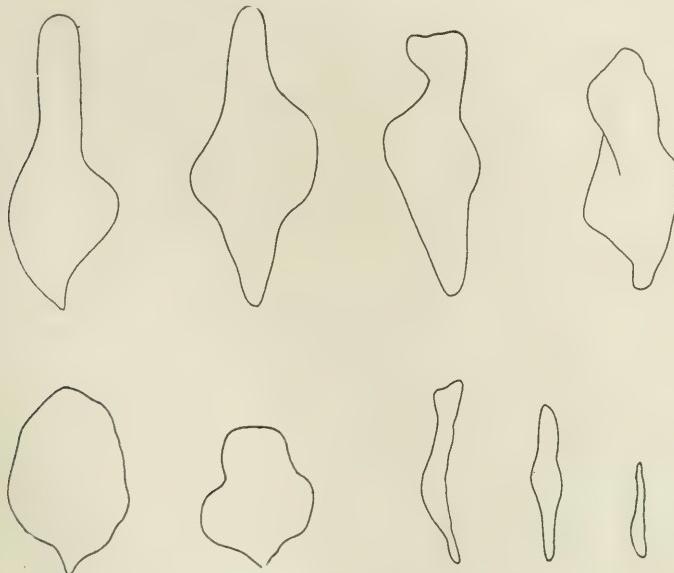


Fig. 1 Nine of the individuals of Experiment 1 which never divided, showing the diversity of form and size among this class. In this figure and in figures 2, 3, and 4, the animals were all drawn by means of the camera lucida, and are all to the same scale. ( $\times 100$ )

As a rule there appeared to be a tendency for these large individuals to gradually decrease in size (as shown by the measurements above given). Figures 2, 3, and 4 show the changes in form and size in certain cases. The forms were frequently nearly normal; sometimes very abnormal, as the figures show. These individuals that never divided were usually black and granular, and often changed shape when transferred to fresh culture fluid, becoming swollen at the anterior end; they later resumed their original form. A cytological study of these animals might be of interest in connection with the 'Kern-Plasm Relation' theory, and with the mechanics of division. Such

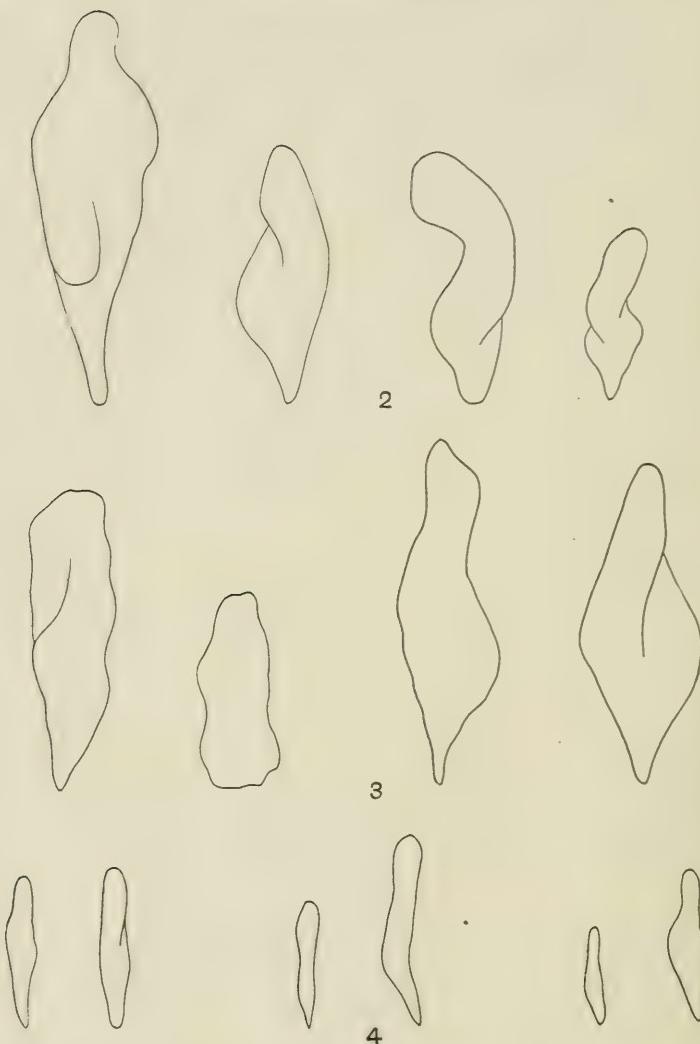


Fig. 2 One of the individuals (15a) of Experiment 1 which never divided, showing the decrease of size and change of form it underwent from December 9 to December 15, inclusive. ( $\times 100$ )

Fig. 3 Two of the individuals of Experiment 1 which never divided, showing the changes of form and size they underwent. The two figures at the left show 16a on December 9 and 11; the other two figures show 110a on December 9 and 13. ( $\times 100$ )

Fig. 4 Three of the individuals of Experiment 1 which never divided. The first two figures show 52a on December 9 and 13; the second two figures show 89b on December 9 and 13; the last two, 19b on December 9 and 11. ( $\times 100$ )

large individuals, never dividing, do not appear after conjugation in all cultures; some later attempts to obtain them for study have not been successful.

#### IV. TYPES OF ABNORMAL RACES

Of chief interest for our purposes are the exconjugants which divided, but produced progeny some of which were deformed. The lines of descent to which these deformed individuals belong I shall call abnormal lines or races; it is they that provide the material for the study of heredity, variation, and selection here set forth.

The abnormal lines differ greatly in the proportion of abnormal individuals produced and in their later history. Not all the individuals produced are abnormal; and not all the abnormal races remain abnormal indefinitely. Reserving many of the details for our section on the results of selection, we may divide these abnormal races into three classes:

1. In the first class are those races which ultimately became normal. There were 39 races of this class. The proportion of abnormal individuals produced ranges in the different races from one per cent to 41 per cent. In these races the abnormal individuals gradually disappear, till finally only normals are produced. Some of the abnormals of this class may be compared with the experimentally deformed animals; and with those which arise rarely in cultures that have not conjugated. They always form a very small proportion of their race, and either die out or produce normal daughter cells. Others however are not strictly comparable with the transient abnormalities described by other workers. They form a considerable and constant proportion of the individuals of their race; are continually produced for several generations; and are often descended from both normal and abnormal sister cells. But through the action of natural conditions and in some cases the selection of the normal individuals, the abnormals become gradually eliminated and the race entirely normal. Data on these 39 races of the three experiments is given in table 2.

TABLE 2  
*The 39 abnormal races which became entirely normal*

EXPERIMENT	RACE	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
1	96a	15	10	314	3	1
	35a	17	10	32	1	3
	14a	13	11	38	3	7
	102a	15	7	17	3	15
	63a	15	7	13	5	28
2	84b	9	5	16	1	6
	49b	9	5	14	1	7
	53a	11	6	25	2	7
	32a	11	8	35	4	10
	63b	11	6	45	6	12
	34a	11	6	14	2	13
	57a	11	5	14	2	13
	29a	11	9	33	5	13
	50b	11	5	12	2	14
	30a	11	7	39	7	15
	40a	9	4	10	2	17
	15a	11	7	17	4	19
	73a	11	9	17	4	19
	56a	11	9	57	13	19
	47a	11	5	16	4	20
	57b	11	9	31	8	21
	92b	22	7	28	8	22
	88a	13	6	13	4	23
	35b	11	8	47	15	24
	12b	11	3	3	1	25
	26a	9	3	3	1	25
	34b	11	4	9	3	25
	54a	11	5	10	4	29
	55b	9	4	10	4	29
	95b	11	3	9	4	31
	99b	9	2	2	1	33
	55a	15	3	4	2	33
	96b	9	4	10	5	33
	85b	9	3	7	4	36
	38a	11	4	10	6	38
	59b	13	7	9	6	40
	16b	15	6	16	11	41
3	52b	17	9	250	2	1
	53b	21	8	212	7	3
Range...		9 to 22	2 to 11	2 to 314	1 to 15	1 to 41
Average..		12	6	38	4	10

Figures 5 and 6 give pedigrees of two of these transiently abnormal races, numbers 14a and 102a, which are fairly typical of this group. Race 14a (fig. 5) was kept for thirteen days. During that time it divided 17 times, giving an average of 1.13 divisions a day. It showed no abnormalities until December 11 (nine days after conjugation), when the three individuals which had arisen from the apparently normal animals selected on December 9 were, one large and very abnormal, one small and abnormal, and the third small and almost normal in appearance. The large one died after having divided once. The two small ones had divided to form four normal individuals on December 13, and on December 15 had given rise to eight perfectly normal animals which were then discarded.

Race 102a (fig. 6) divided very slowly at first, averaging 0.5 divisions a day. The average division a day for the whole time the race was kept was 0.8, the animals dividing much more rapidly toward the last. This exconjugant showed no signs of being abnormal until December 11, when the three forms present were very abnormal, two being doubles and one a very much swollen individual. One of the double forms moved in a circle so swiftly that it could not be drawn. Both of the double forms died without dividing; the large swollen one gave rise to a perfectly normal race which was discarded on December 17.

2. In the second class of abnormal races are those which persistently produced abnormal individuals throughout their history. There were 9 such races in Experiment 1 and 88 in Experiment 2. They differed greatly in the proportions of abnormals produced, varying from races 100 per cent abnormal to those but 3 per cent abnormal. The pertinent data for these 97 persistently abnormal races is given in table 3.

In many of these persistently abnormal races individuals appeared which were normal in form. But these if propagated eventually produced abnormals. Further details as to this will be given in our section on the effects of selection.

Figures 7 and 8 give pedigrees of two of the short-lived races of this group, 39b and 40b, which show the typically persistent

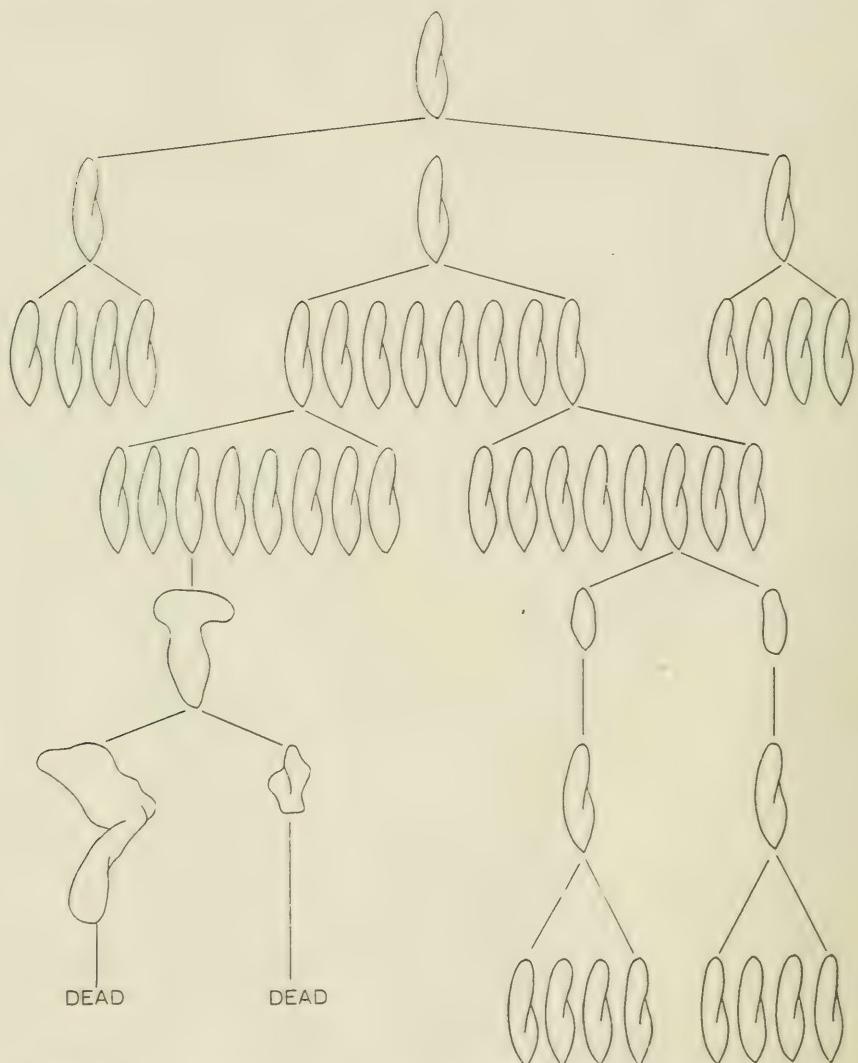


Fig. 5 Pedigree of 14a, one of the abnormal races which became entirely normal.  $\times 100$

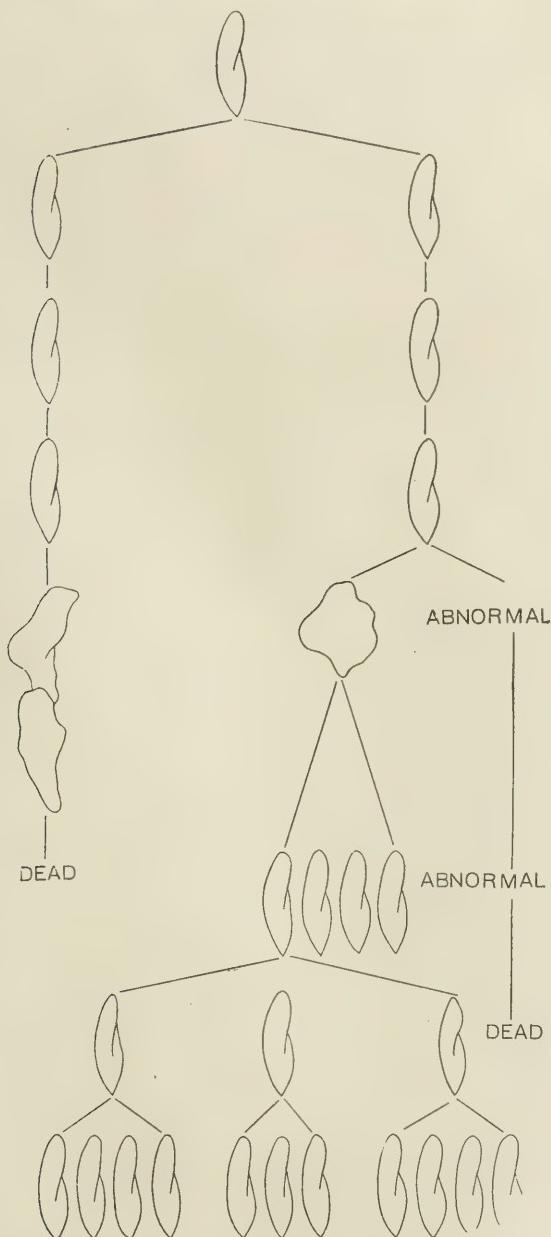


Fig. 6 Pedigree of 102a, one of the abnormal races which became entirely normal. On some dates the abnormal forms could not be drawn; they are simply designated as 'abnormal.'

TABLE 3  
*The 97 races which remained persistently abnormal*

EXPERIMENT	RACE	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
1	2a	15	3	3	1	25
	16b	9	5	18	2	10
	25b	9	2	0	2	100
	39b	25	9	14	12	46
	40b	17	8	10	8	44
	47a	9	3	0	5	100
	91a	15	2	0	2	100
	106a	9	2	0	2	100
	C	191	303	2027	2683	57
2	2b	27	11	55	19	26
	6a	11	4	5	4	44
	7b	15	6	4	9	69
	8a	17	5	6	7	54
	66b	11	6	4	13	76
	8b	17	7	20	15	43
	9a	11	4	2	6	75
	99b	11	2	2	11	33
	11a	21	10	13	28	68
	11b	21	8	21	14	40
	14b	15	6	17	8	32
	16a	23	7	25	8	24
	18b	11	6	18	5	22
	19a	17	6	21	16	43
	19b	23	6	10	16	66
	20a	27	19	224	187	45
	20b	19	5	11	10	48
	21a	21	10	25	17	40
	21b	13	4	2	5	71
	22a	13	4	6	3	33
	22b	11	4	6	6	50
	23a	25	8	21	5	19
	23b	25	8	33	25	43
	24a	9	2	2	1	33
	24b	11	6	10	4	29
	25a	23	7	13	10	43
	25b	11	4	7	4	36
	27a	11	4	4	5	56
	27b	27	27	303	556	65
	28a	15	5	19	9	32
	28b	13	4	7	8	53
	29b	19	8	26	18	41

TABLE 3—Continued

EXPERIMENT	RACE	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
30b		19	8	18	17	49
31a		13	5	5	11	69
31b		11	5	13	8	38
32b		11	2	3	1	25
33a		21	5	12	5	29
33b		11	5	5	6	55
35a		11	5	4	11	73
39a		13	4	3	4	57
39b		23	8	36	20	36
43a		27	10	322	22	41
43b		11	5	13	6	32
44a		11	4	8	2	20
44b		13	2	2	2	50
46b		11	4	11	5	31
48a		27	19	91	133	59
48b		23	7	28	9	24
54b		13	7	27	3	10
56b		9	4	4	8	66
58a		17	8	35	4	10
58b		11	3	3	4	57
59a		11	6	30	1	3
61a		13	5	25	7	22
62b		11	4	4	6	60
63a		11	5	22	12	35
65a		17	6	10	9	47
67a		13	4	11	6	35
67b		21	6	15	11	42
68a		19	7	31	4	11
68b		11	2	4	1	20
69b		17	8	24	11	31
70a		21	7	23	41	64
70b		27	4	18	19	51
71a		13	5	8	7	47
72a		15	7	20	10	33
73b		11	5	7	10	59
74a		17	7	12	32	73
74b		11	6	6	26	81
75a		11	3	5	3	37
75b		11	5	13	4	24
76a		11	4	9	8	47
76b		13	4	9	2	18
79a		27	21	186	143	43
82a		9	3	3	6	67

TABLE 3—Continued

EXPERIMENT	RACE	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
2	82b	9	4	4	8	67
	84a	27	7	15	20	57
	89a	11	5	10	66	37
	89b	27	7	45	22	33
	90a	19	4	11	4	27
	90b	21	4	8	4	33
	92a	17	3	7	5	42
	94a	23	7	12	23	66
	94b	13	3	7	5	42
	96a	23	7	18	25	58
	98a	15	5	8	2	20
	99a	13	4	5	3	37
	100b	19	3	6	5	45
Range...		9 to 191	2 to 303	0 to 2027	1 to 2683	3 to 100
Average		18	9	41	47	53

appearance of the character in all their lines. Race 39b (fig. 7) divided seven times in eight days, forming only normal individuals until December 11, when six of the eight individuals present were very much misshapen. After this date only abnormalities were produced by this race, which died out on December 27. There was a very large proportion of double forms among the individuals of this race, eleven of the twenty-five abnormalities produced being of this character.

Race 40b (fig. 8) divided regularly to form only normals up to December 11, when three out of the five then present were abnormal. The two normals died out and the race was carried on through the abnormal individuals. These produced only abnormal progeny and the race died out on December 19.

Race C, described on pages 414 to 420, is a typical long-lived race of this group.

3. As the third group of abnormal races we may distinguish those which did not become entirely normal, but from which normal races were obtained by continued selection. This group will be dealt with in our section on selection.

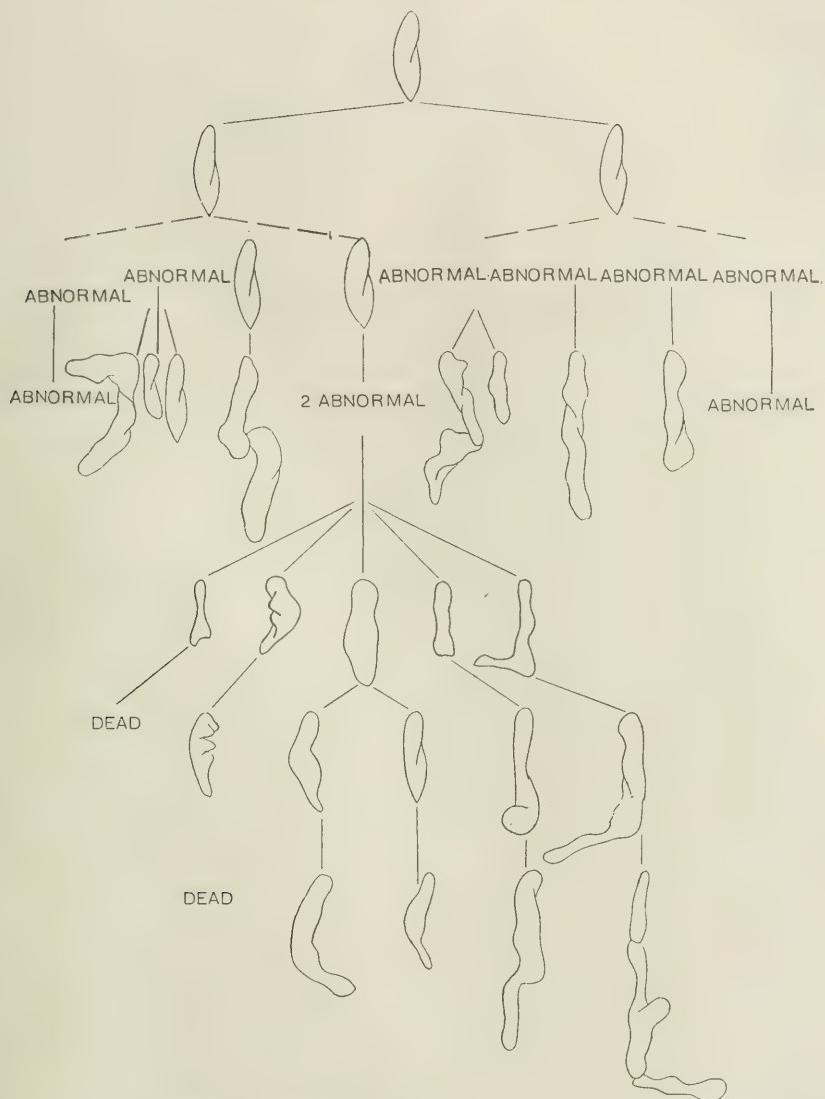


Fig. 7 Part of the pedigree of *39b*, one of the short-lived races which remained abnormal throughout its life. The broken connecting lines designate the omission at that point of two entirely normal generations.

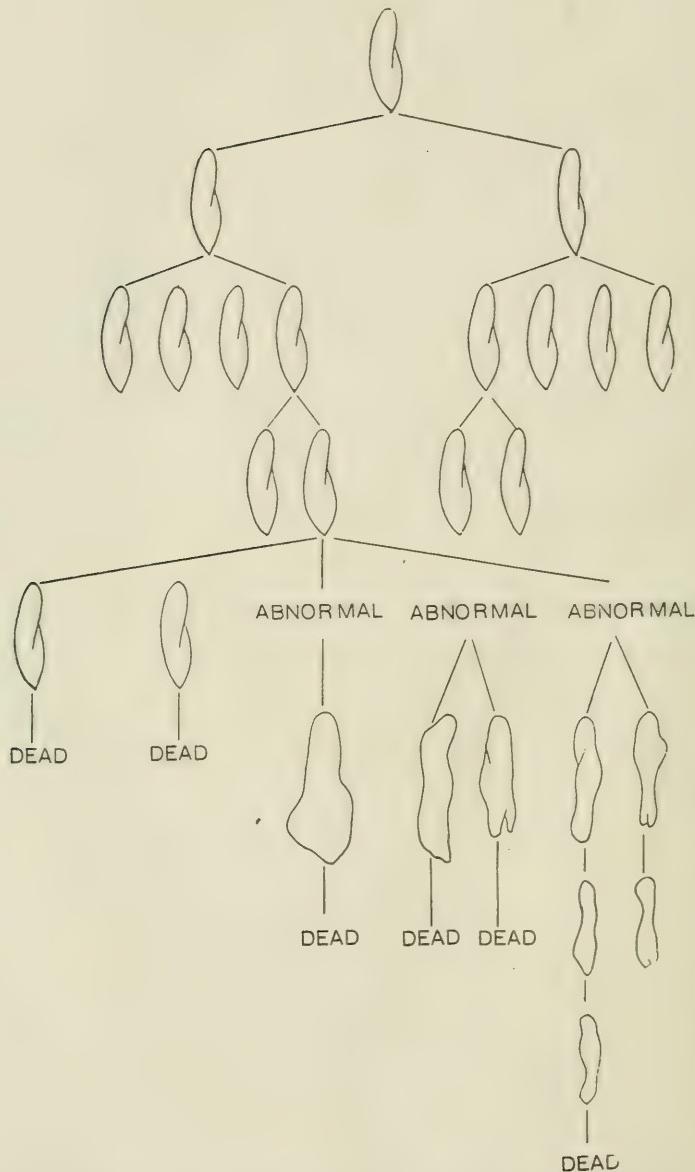


Fig. 8 Pedigree of 40b, another short-lived race which was abnormal throughout its life.

## V. NATURE OF THE ABNORMALITIES

Descriptively considered, the abnormalities shown by the individuals of the abnormal races are of many diverse sorts. I have been able to distinguish 40 different types of abnormals. These are shown in figure 9, and their frequencies in the three experiments are given in table 4. Most of the races of Experiment 1 developed one or more representatives of a large number of these types; some of the races had representatives of all but two or three. The high frequencies of so many of these types in Experiment 1, as given in table 4 shows how great the diversity of form is among the individuals of that group. In experimentally produced abnormalities reported by other workers on other animals, this diversity of type is always shown; as Mall ('08) says, there is never found any precise type of abnormality characteristic of certain conditions, but only a general character, a tendency to abnormality of many different types. But some races of my experiments were more or less characterized by a few types, which I have called their predominant types. The best examples of this predominance of type are race C of Experiment 1, and races 56a, 56b, and 59b of Experiment 3. During the early part of their histories these races showed considerable diversity of abnormal type; but this diversity was rapidly reduced and the abnormals became limited to a few types in all four of these races. During its whole history race C produced in its observed cultures 2683 abnormals; 1331 or 50 per cent of these were of type 33; 945 or 35 per cent were of type 27. The predominant type of the other three races was the same, number 27 of figure 9. Race 56a produced 1428 abnormals; 79 per cent of them were of that type; 59 per cent of the 780 abnormals of 56b were of type 27; and 50 per cent of the 2096 abnormals of race 59b were of the same type.

In the abnormals of Experiment 2 there was considerably less diversity of type than in the abnormals of Experiment 1. Only 30 of the different types were observed in Experiment 2; most of these appeared only a small number of times, 90 per cent of the abnormals being of five types (11, 26, 27, 28, 30). Table 4 gives

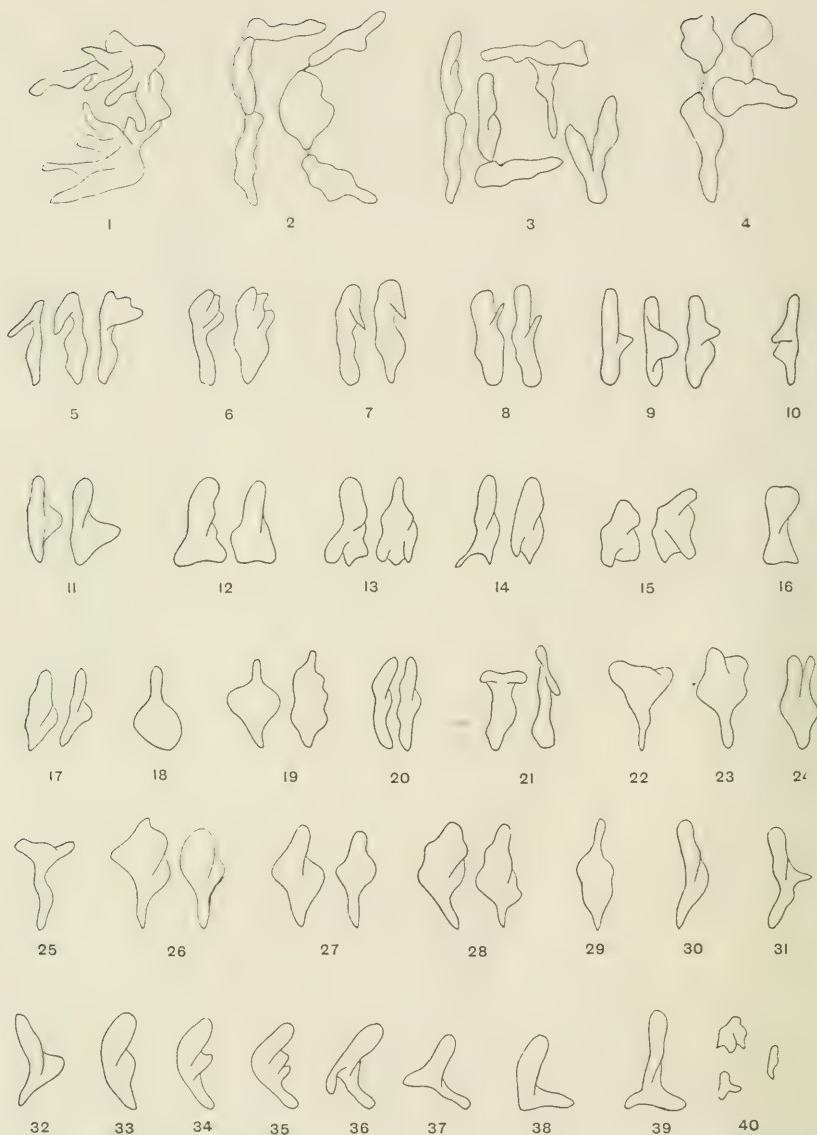


Fig. 9 The 40 types of abnormalities observed in the three experiments.<sup>7</sup> In some cases two or more examples of one type are given in order to convey some idea of the variation within a type. The types which appeared in the three experiments and their number and proportion are given in table 4.

the numbers of the thirty types that appeared among the abnormalities of this experiment and their frequencies.

Likewise in the abnormalities of Experiment 3 there was less diversity of type than in those of Experiment 1. Table 4 gives the numbers of the 27 types observed and their frequencies; 91 per cent of all the abnormalities of Experiment 3 were of five types (22, 26, 27, 30, 39).

The lessened diversity of type observed in the experiment with the members of a pure line (Experiment 2) as compared with the members of a wild culture (Experiment 1) is just what we might expect from the constitutions of the two groups. The wild culture probably contained many very diverse stocks; the conjugants probably were widely different in their gametic constitutions; so that there is the greatest possible opportunity for variation among their progeny. But all the members of the clone used in Experiment 2, by the theory of the pure line, have identical gametic constitutions. This may be and probably is highly heterozygous; but the diversity possible to the progeny of the different conjugants is limited by this heterozygosity. In Experiment 1 the diversity is limited only to the characters possible to 262 members of the species *Paramecium caudatum*, since every conjugant might possibly be different from every other; in Experiment 2 however this diversity is limited to the characters possessed by one individual *Paramecium* (the progenitor of the pure line used) and their possible recombinations. This fundamental difference in the constitutions of the two groups is very probably a cause of the production of greater diversity in form, and in the size, shape, and length of life of the individuals that never divided, mentioned on page 393.

The individuals used in the third experiment cannot be considered in this discussion, since they were derived from a small culture kept for some time in the laboratory, and the history of only 28 exconjugants is known.

Most of the abnormalities which have been described in the metazoa are explained as arrests in development; as suppressions of some part of the normal process of growth and differentiation. Some of my abnormalities answer to this description;

TABLE 4  
*Showing the frequencies of the different types of abnormalities in the three experiments*

TYPE	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		TOTAL	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1	75	1.36	2	0.05	6	0.10	83	0.56
2	31	0.56			3	0.05	34	0.23
3	436	7.92	32	0.90	90	1.61	558	3.81
4	26	0.47			10	0.18	36	0.24
5	102	1.85	4	0.11	88	1.58	194	1.32
6	20	0.36					20	0.13
7	337	6.12	8	0.22	6	0.10	351	2.39
8	112	2.03			1	0.01	113	0.77
9	55	1.00	9	0.25	4	0.07	68	0.46
10			22	0.61	2	0.03	24	0.16
11	197	3.58	109	3.06	134	2.41	440	3.01
12	107	1.94	9	0.25			116	0.79
13	41	0.74	3	0.08	32	0.57	76	0.52
14	123	2.23	11	0.31	42	0.75	176	1.20
15	23	0.41	1	0.03	6	0.10	30	0.20
16	4	0.07	17	0.47	15	0.27	36	0.24
17	202	3.67	25	0.70			227	1.55
18	31	0.56	5	0.04			36	0.24
19	22	0.40	11	0.31	5	0.09	38	0.26
20	22	0.40	3	0.08	10	0.18	35	0.24
21	43	0.78					43	0.29
22	43	0.78	2	0.05	322	5.78	367	2.51
23	25	0.45	5	0.14			30	0.20
24	30	0.54					30	0.20
25	17	0.31					17	0.12
26	278	5.03	384	10.76	2891	51.94	3553	24.28
27	1028	18.69	919	25.76	682	12.25	2629	17.97
28	83	1.51	102	2.86	2	0.03	187	1.27
29	9	0.16			8	0.10	17	0.12
30	211	3.83	1688	47.32	373	6.70	2272	15.52
31	36	0.65	9	0.25			45	0.31
32	1	0.02	54	1.51	2	0.03	57	0.39
33	1430	26.00	87	2.44	54	0.91	1511	10.32
34	56	1.02	15	0.42	11	0.20	82	0.56
35	13	0.23			11	0.20	24	0.16
36	80	1.45	1	0.03			81	0.55
37	1	0.02	9	0.25			10	0.06
38	55	1.00					55	0.37
39	1	0.02	5	0.04	736	13.22	742	5.07
40	93	1.69	16	0.45	20	0.35	129	0.88
Total.....	5499	37.58	3567	24.38	5566	38.04	14632	100.00

certainly the individuals that never divided, the double and the monster forms, and those abnormal races in which the division rate is very much lowered, can be explained in that way. But in a few of the abnormal races the division rate was normal, and there was very little tendency to die out; indeed they showed no abnormal characters at all except the development of a slightly bizarre form. Race C is a good example of such a race. It lived for 191 days, had an average division rate of 1.16 per day, and was consistently abnormal throughout its history.

The mortality of the majority of the abnormal races was however considerably greater than that of the normal races. As the latter were all discontinued within a few days after conjugation, nine days being the usual length of time they were kept, no exact comparison can be made between their mortality and that of the abnormal races. However, they may be compared in a few ways, that show there are differences between them in vitality and tendency to die out. In each of the three experiments a large proportion of the abnormal races died within two weeks after conjugation, only a few races being kept for any length of time. In Experiment 1 there were 21 abnormal races; 14 per cent (3) of these lived for over a hundred days (105, 131, 191); 29 per cent were lost or discontinued after, on the average, 9 days of life; 12 (57 per cent) died after an average length of life of 15 days, and an average number of generations, 6. This gives an average division rate of 0.4 a day. The normals, discontinued 9 days after conjugation, had an average division rate of 1.12 a day. The facts in the other experiments are very similar to these; it is evident in all three that the abnormal races have, as a rule, a lower vitality and a greater degree of mortality. I explain this as largely due to the fact that the abnormal forms are hindered in their locomotion by their abnormal shape and so have not the normal facilities for meeting the exigencies of their existence. They often lie motionless on the bottom of the dish or slide for long periods; it was this lack of energetic movement which made possible camera lucida drawings of so large a proportion of them. It is probably for this reason that so few abnormalities are found in the usual culture.

## VI. THE ABNORMALITIES AS HEREDITARY CHARACTERS; VARIATION, INHERITANCE, AND SELECTION

As we have seen, the abnormalities which we are considering arise in consequence of conjugation. If half of a given stock are allowed to conjugate, the other half not, the former develops many of these abnormal races, while the latter develops none. This shows that the abnormalities cannot be considered due to infection, nor their reappearance in the stocks to the handing on of an infecting organism.

Since some lines are quite without abnormalities, while in others, under the same conditions, the abnormalities reappear for generations, it is clear that the tendency to abnormality is inherited. That is, the difference between a stock that thus produces abnormal individuals and one that does not, lies in the constitutions of the stocks themselves, and is something that is transmitted during vegetative reproduction.

Such hereditary diversities occur not only between normal and abnormal stocks, but also among the abnormal stocks themselves. Precise types of abnormality are indeed not inherited exactly from parent to progeny; within a given line as we have seen there is great variation as to whether abnormality appears at all in a given individual, and as to the precise kind that occurs when the individual is abnormal. Nevertheless, as before set forth, certain types of abnormality are particularly common in some lines, other types in other lines. The diverse lines differ hereditarily in respect to something of which the diverse typical abnormalities are the outward results. It is only by keeping in mind the characteristic differences between lines that we shall be able to grasp their relation to the problems of heredity.

The hereditary diversities thus far mentioned are between lines derived from diverse exconjugants. If anything like Mendelian inheritance occurs in infusoria, we could well expect such lines to show hereditary differences; this is of course as a rule true in any organism after the union of two parents to produce progeny.

On the other hand, in vegetative reproduction, and in general in long continued uniparental reproduction of any sort, a remark-

able constancy in hereditary characteristics has been generally reported. All the progeny thus coming from a single parent have seemed uniform in their hereditary characteristics, though they may differ in their bodily appearance. And this is quite in agreement with the known cytological processes accompanying the two types of reproduction. In biparental reproduction there is a reduction and recombination of the nuclear elements, of precisely the same sort as the variation and recombinations of characters in the progeny in Mendelian inheritance. In uniparental reproduction, particularly of the vegetative kind,<sup>2</sup> such nuclear reductions and recombinations are not known; and the uniformity of the progeny is in agreement with this.

These relations, with others not necessary to recount here, have given origin to the conception of the genotype as the hereditary constitution, in contradistinction to the bodily appearance. The genotype is commonly held not to change in vegetative reproduction, or but rarely, and then by marked sudden steps, or mutations. In biparental reproduction the genotype does indeed change, but seemingly by mere shiftings and recombinations, in numerically predictable ways; so that the relations here are quite in agreement with the condition sketched above for uniparental reproduction.

A somewhat rigid, stereotyped scheme of heredity naturally results from the view of the facts as just set forth; in particular, evolution by gradual change, guided by natural selection, appears to be excluded. This becomes still more marked if we conclude with Bateson ('14) that all mutations consist in the dropping out of factors. However, certain investigators in genetics oppose this rigid view, holding that, over and beyond Mendelian recombinations, hereditary variations of slight degree are frequently occurring, so that evolution may well be continuous and guided by selection. The recent papers of Castle give typical expression to this point of view.

<sup>2</sup> In their recent description of endomixis during the vegetative reproduction of *P. aurelia*, Woodruff and Erdmann observed neither reduction nor fusion of nuclear elements.

If hereditary variations are frequently occurring, aside from Mendelian recombinations, it should be possible to find them in vegetative reproduction. Here we are freed from the mixing of types which makes these relations so difficult to interpret in biparental reproduction. The hereditary abnormalities with which the present study deals seem to offer a favorable opportunity for the study of this matter. Within the same line of vegetative descent we find individuals that are in appearance normal, others that are outwardly abnormal. Can we by continued selection of normal individuals on the one hand, of abnormal individuals on the other, break our single stock into two or more, differing in hereditary constitution?

#### *Experiments in selection*

To answer the question just proposed, selection was carried on for many generations in a considerable number of abnormal stocks.

As before set forth, some of the races in which abnormalities occurred gradually changed character and became entirely normal. In other races both normals and abnormals appeared for long periods, giving opportunity for long continued selection. We will first take up the large race C, of Experiment 1.

Figures 10 and 11 give extracts from the pedigree of race C. This race, derived from exconjugant 101a, of Experiment 1, was kept for 191 days and produced during that time 4710 individuals in the observed cultures; 2683 of these were abnormals. The early history of this race is shown in chart 1.

CHART 1										
Early history of Race C										
(n = normal; ab = abnormal)										
December 2	5	7	9	11	13	15				17
1n—1	4n—4	8n—2	8n—1	4n—1	{ 8n —1 4n —1		Discontinued			
					{ 1ab—1 1ab—1		Dead			
					{ 1ab—1 1ab—1		Race C			

In this and in the succeeding charts the first figure under each date shows the number of animals present in that line on that date; *n* means that these were normal; *ab* means that they were abnormal. The second figure, following the dash, denotes the number chosen to carry on the slide line, the others being put into

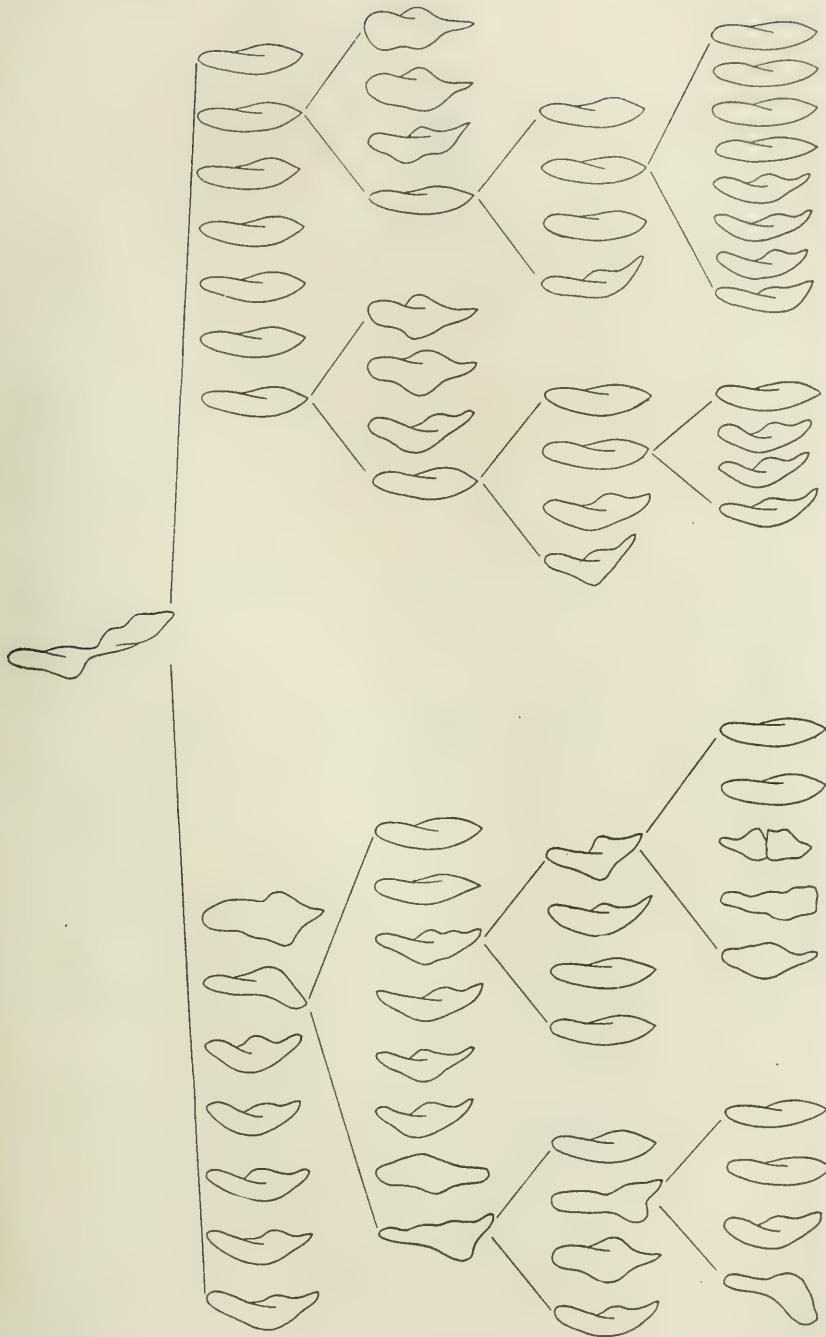


Fig. 10 Extract from the pedigree of Race C, showing on the left side a line in which abnormals were selected; on the right side a sister line in which normals were selected. The persistent appearance of the abnormalities in both lines, regardless of the selection, is evident.

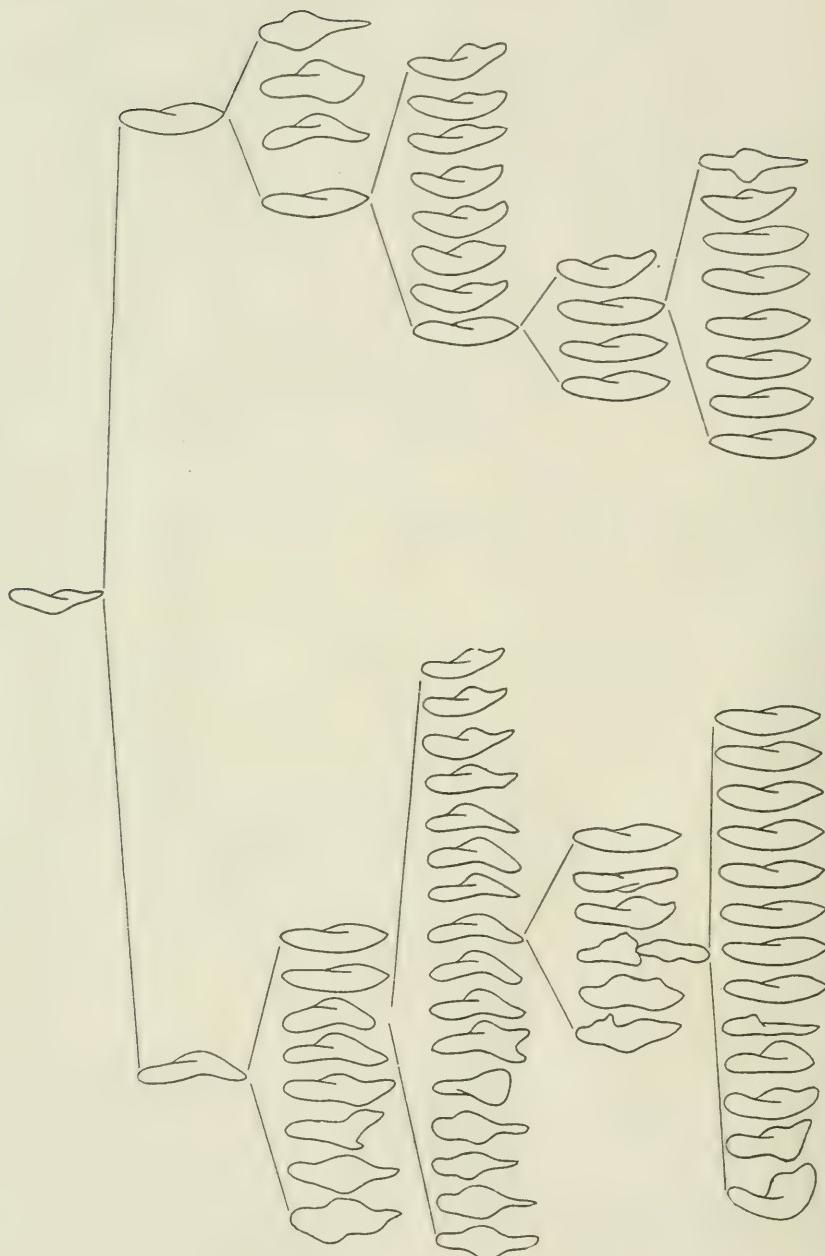


Fig. 11 Same as figure 10, but from a different group of lines.

bottle cultures, or discarded. In a number of the charts the second figure is omitted since the selection was the same on every date, one animal of a particular kind being always chosen. When both normals and abnormals are present in a single line (Chart 2) the kind which are entirely discarded are placed above, the kind from which one is chosen is placed below, each with its number. *D* means that on that date that line died out. Chart 1 therefore reads: On December 2 the normal exconjugant was isolated; December 5, it had divided to form four normals, all of which were kept; December 7, these had divided into eight normals, two of which were kept; these two had divided on December 9 to eight normals, one of which was kept; December 11, this one had given rise to four normals, one of which was kept; December 13, this one had given rise to two abnormals, both of which were kept, and to eight normals, one of which was kept; December 15, the normal had divided to form four, one of which was kept, and the two abnormals, neither of which had divided, were both kept; December 17, the normal line was discontinued entirely, one of the abnormal had died, and the other was kept and gave rise by repeated divisions to all the later individuals belonging to Race C.

Race C therefore appeared to be entirely normal for eleven days after conjugation, at the end of that time producing two abnormals, one of which was double, the other small and deformed. This small one gave rise to all the later 4681 observed individuals of this race, comprising 303 generations. In 34 lines of this race a continuous selection of normals was made, in one case for 32 days and 24 generations. The data from these lines is given in table 5. Their character was not changed in any way by this selection; the average proportion of abnormality for these 34 lines is only 2 per cent less than that of the race as a whole (table 3). In one line only normals were produced for as long as six days, the line at the end of that time again producing abnormals. The history of this line with a few of the others is given in chart 2. In every case one normal was selected each

TABLE 5  
*Data from the 34 normal-selected lines of Race C*

GROUP	NUMBER OF LINES	NUMBER OF DAYS SELECTION CARRIED ON	NUMBER OF GENERATIONS OF NORMALS PRODUCED	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
2	5	14	8	36	33	48
3	29	32	24	233	294	56
Total	34			269	327	55

day to carry on the line. It is evident that this selection has had no effect on the character of these lines.

CHART 2

History of six normal-selected lines of Race C  
(*n* = normal; *ab* = abnormal; *D* = died out)

April	May													
24	26	28	30	2	4	6	8	10	12	14	16	18	20	22
					6ab	1ab								
					10n	3n	D.							
					1ab	7ab								
					3n	6n	D.							
					9ab	2ab	5ab							
					7n	8n	2n	D.						
					10ab	6n	D.							
					1ab	6ab								
					3n	2n	D.							
					20ab	2ab		2ab	3ab	3ab	2ab	2ab		
					12n	4n	2n	1n	2n	1n	1n	6n	2n	

In 43 lines of race C a continuous selection of abnormalities was made, in one line for 40 days and 37 generations. Their character was not changed by this sort of selection; their proportion of abnormality is the same as that for the race as a whole (table 3). The data from them is given in table 6, and the histories of a few in chart 3. In very case one abnormal was chosen on each day to carry on the line.

CHART 3

(n = Normal; ab = abnormal; D = died out)  
History of five of the abnormal-selected lines of Race C

May	10	12	14	16	18	20	22	24	26	28	30
				2n	1n			2n	2n		
				2ab	3ab	4ab	2ab	2ab	2ab	D.	
				4ab	2ab	4ab	4ab	1ab	1ab	D.	
						4n		4n	2n		
				2ab	3ab	4ab	3ab	4ab	2ab	2ab	D.
					3n	1n	2n				
				2ab	3ab	3ab	2ab	8ab	4ab	D.	
						3n	1n	3n			
6ab		1ab	3ab	2ab	4ab	2ab	3ab	1ab	2ab	D.	

Three times in the history of this same race all of the individuals died with one exception; in every case this single sur-

viving individual was an abnormal animal which gave rise by repeated divisions to the further representatives of its race. On two of these occasions the two groups of lines which rose from the two first daughter cells of this single abnormal animal were treated differently; in one group the most normal individuals were selected; in the other group, sister to the first, the most abnormal were selected. The data for these two pairs of groups is given in table 7. In this table, one abnormal-selected line in the first set lived longer than 56 days; in the second set one normal-selected line lived longer than 20 days; both however for purposes of comparison are counted in this table as having died out at the same time with the other lines of their sets.

TABLE 6  
*Data from the 43 abnormal-selected lines of Race C*

GROUP	NUMBER OF LINES	NUMBER OF DAYS SELECTION CARRIED ON	NUMBER OF GENERATIONS OF NORMALS PRODUCED	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
1	3	40	30	58	83	59
2	9	36	19	103	126	55
3	25	32	31	135	163	55
4	6	18	13	49	93	65
Total	43			345	465	57

TABLE 7  
*Data from two sets of sister groups of lines; in one group of each set the most abnormal were selected; in the other group, the most normal*

SET	GROUP	NUMBER OF LINES	NUMBER OF DAYS SELECTED	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
1	Abnormal-selected	15	56	48	387	489	56
	Normal-selected						
2	Abnormal-selected	20	20	25	131	157	54
	Normal-selected						

With these two exceptions, one abnormal-selected and the other normal-selected, the two groups in both sets are very much alike. They show a great deal of similarity in length of life, number of generations, and proportion of abnormality, the diverse selection having had no effect on the character of the lines. Indeed, in both sets, the lines of the normal-selected group show a larger proportion of abnormals.

Thus in line C we have a race in which long continued selection has no effect on the inheritance of abnormalities. And these results are typical of a large number of races—all those classified on page 399 as class 2 (97 races all together). In all of these races where such a procedure was possible the selection of normals was carried on as long as the race lived, in an attempt to establish a normal line; but with all these races this effort failed. The normals selected continued indefinitely to produce abnormal progeny in the original proportions. With these races therefore the results of continued selection are the same as those obtained by the majority of investigators in uniparental reproduction: selection does not alter the inherited constitution of the line nor produce two genotypes from one.

In another set of abnormal lines however, different results were reached. In twenty-five races of our three experiments the single race was split up by selection into hereditarily diverse groups, one group composed of lines that continued to produce abnormals, the other composed entirely of normals. The main facts as to these twenty-five races are given in table 8. Many of these lines were kept but a short time, so there might be doubt as to the precise significance of the results from them. Certain lines however were kept for a very large number of generations and give conclusive results. This was the case with the lines *A* and *B* of Experiment 1, and with the six lines of Experiment 3. Some details will therefore be given as to these races.

The history of race *A* may be taken as a type. The exconjugant from which this arose gave rise to normal individuals only for seven days, or until December 9, when eight abnormals and ten normals were found on the slide. After this the race did

TABLE 8 (first part)

*Data from the 25 races of the three experiments which were affected by selection*

EXPERIMENT	RACE	ABNORMAL LINES					Per cent abnormal
		Number	Days	Generations	Normals	Abnormals	
1	48a	3	27	9	27	6	18
	53b	1	11	3	2	2	50
	93b	4	23	10	38	9	19
	105b	1	17	2	1	1	50
	A	178	131	74	524	850	63
	B	314	105	83	1328	1788	57
2	2a	1	15	4	2	7	78
	4a	2	23	5	6	7	54
	4b	4	27	14	52	22	30
	7a	2	27	15	34	19	36
	10a	1	13	4	8	3	27
	10b	24	27	26	293	323	52
	18a	20	27	28	634	748	54
	40b	1	17	6	6	2	25
	41a	1	11	4	6	9	60
	62a	1	11	5	5	3	38
	69a	9	27	25	172	213	55
	83b	7	27	15	40	22	35
	87a	7	27	15	55	23	29
	Total...	733			16411	8341	
Average		29	40	26	656	334	39

not return to complete normality, but always showed a large proportion of abnormal forms among its members. For some time all the normal individuals were discarded and all the abnormalities kept. By December 23 this procedure had resulted in so many lines (178) that all but 45 of the most abnormal were discarded. These 45 very abnormal lines were grouped for convenience into 13 categories. The genetic history of the lines

TABLE 8 (second part)

*Data from the 25 races of the three experiments which were affected by selection*

EXPERIMENT	RACE	X*	NORMAL LINES					Per cent
			Number	Days	Generations	Normals	Abnormals	
1	48a	7	1	6	7	20		
	53b	7	1	5	7	7		
	93b	9	4	6	5	50		
	105b	1	1	5	2	4		
	A	62	6	26	22	134		
	B	5 to 49	3	20	21	126		
2	2a		1	4	8	31		
	4a		1	6	3	6		
	4b	2	1	6	4	77		
	7a	8	1	4	3	7		
	10a	8	1	4	3	10		
	10b	4	1	4	3	8		
	18a	18	4	6	9	75		
	40b		1	8	3	8		
	41a	4	1	8	3	8		
	62a	6	1	6	4	16		
	69a	8	2	4	6	50		
	83b		1	12	4	6		
	87a	8	4	12	6	53		
3	54b	9 to 42	15	35	20	3589		
	55b	9 to 11	2	12	13	272		
	56a	9 to 50	8	37	24	936		
	56b	9 to 44	11	24	26	1164		
	59a	9 to 37	2	47	35	425	3	0.5
	59b	37 to 53	12	39	27	1334		
Total...			86			8346	3	
Ave.....		24	3	14	11	334	0.12	0.2

\* X is the number of days that the selection of normals went on before the normal lines were isolated.

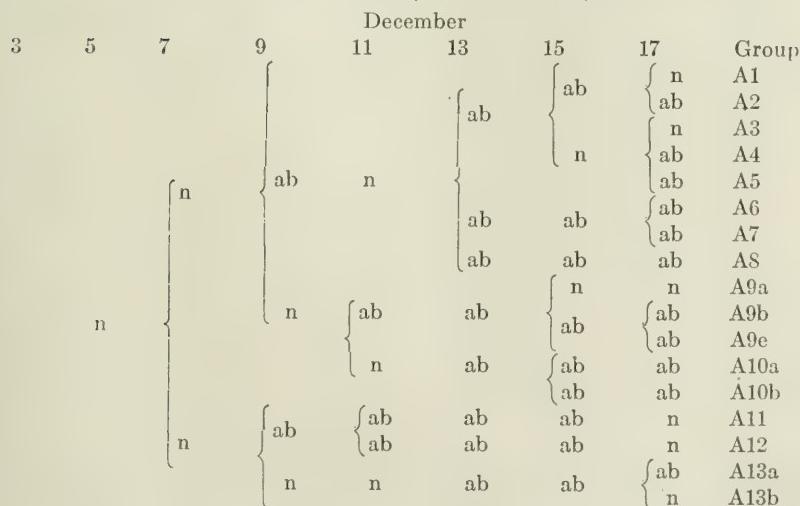
which made up these groups is given in chart 4. Two of these groups of lines, A2 and A6 died very soon; the other lines lived for some time and the data from them is given in table 9.

TABLE 9  
*Data from eleven groups of Race A*

GROUP	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMAL
A1.....	31	16	22	35	61
A3.....	69	40	34	85	71
A4.....	37	22	13	44	77
A5.....	131	64	61	182	75
A7.....	33	16	18	54	75
A8.....	47	16	14	55	80
A9.....	69	30	35	154	81
A10.....	25	11	31	19	48
A11.....	27	11	13	26	67
A12.....	51	13	26	54	67
A13.....	115	74	391	132	25
Largest.....	131	74	391	182	81
Total.....			658	850	56
Average.....	58	28	60	77	56

CHART 4

## Genetic history of the thirteen groups of Race A ( $n$ = normal; $ab$ = abnormal)



After December 23 every one of the lines belonging to race A was carried on in two parts or sub-lines; in one sub-line the most normal individuals were selected; in the other sub-line the most abnormal individuals were selected. This procedure kept going a number of normal-selected lines up to as many as 40; and an equal number of abnormal-selected lines. In almost all of the normal-selected lines the continuous selection of normals was not possible; some generations were entirely abnormal, no normals at all being produced. But 62 days after this normal selection had begun, one normal was isolated which gave rise to six lines in which the continuous selection of normals was possible for some time before they died out. Their history is given in chart 5, and the data afforded by them in table 10. The largest of these six lines was kept for 26 days after the continuous selection of normals was begun and gave rise to 22 generations of normals. There were 42 individuals in the observed cultures of this one line; one of these was abnormal; all the rest were normal.

TABLE 10

*Data from the six lines of Race A in which the selection of normals was continuous*

SELECTION CONTINUOUS					LINES ENTIRELY NORMAL		
Days	Generations	Normals	Abnormals	Per cent abnormal	Days	Generations	Individuals
12	10	21	0	0	12	10	21
10	10	12	0	0	10	10	12
6	6	8	0	0	6	6	8
22	18	32	1	3	10	7	11
14	13	20	0	0	14	13	20
26	22	41	1	2	20	15	25
Greatest							
26	22	41	1	3	20	15	25
Total		134	2	1			97

As all the animals of these six lines were descendants of one normal individual present on February 27 (chart 5), they can all be counted together as one group, which will give 134 normal

individuals to 1 abnormal individuals in 21 generations. This is a very low proportion indeed as compared with that for the race as a whole (56 per cent). Moreover, every one of these six races was entirely normal for some time before it died, as

## CHART 5

Genetic history of six lines of Race A in which the selection of normals  
was continuous  
( $n$  = normal; D = died out)

Line	Feb.	Meh.	27	1	3	5	7	9	11	13	15	17	19	21	23	25	27	
*					4n	$\left\{ \begin{array}{l} 3n \\ 4n \\ 2n \\ 4n \end{array} \right.$				D.								
A13b1	1n	4n			4n	$\left\{ \begin{array}{l} 4n \\ 4n \\ 4n \\ D. \end{array} \right.$												
						$\left\{ \begin{array}{l} 2n \\ 4n \end{array} \right.$				1ab								
						3n	$\left\{ \begin{array}{l} 2n \\ 4n \\ 2n \\ 2n \end{array} \right.$				5n	4n	2n	4n	4n	D.		
							$\left\{ \begin{array}{l} 7n \\ 1n \\ 4n \\ 4n \end{array} \right.$				4n	8n	4n	4n	2n	2n	1n	D.

shown in the table. Figure 12 gives that part of the pedigree of race A which includes these six lines.

In 24 of the abnormal selected lines abnormalities were produced in every generation for some time and their selection was therefore continuous. The history of six of these lines is given in chart 6. Table 11 gives the data from all of them. The largest line was kept for 36 days after the selection of abnormalities became

## CHART 6

Genetic history of six of the lines of Race A in which the continuous selection of  
abnormals was made  
( $n$  = normal; ab = abnormal; D = died out)

December												January					
21	23	25	27	29	31	2	4	6	8	10	12	14	16	18			
2n			1n														
2ab	2ab	2ab	1ab	3ab	1ab	1ab	1ab	1ab	1ab	D.							
1n																	
1ab	2ab	1ab	1ab														
1n																	
1ab	1ab	1ab	1ab														
2n																	
2ab	3ab	1ab															
1ab	1ab	4ab	1ab	2ab	2ab	2ab	2ab	1ab	2ab	4ab	2ab	2ab	1ab	D.			
1n	1n																
1ab	1ab	1ab	1ab	1ab	1ab	1ab	1ab	1ab	1ab	D.							

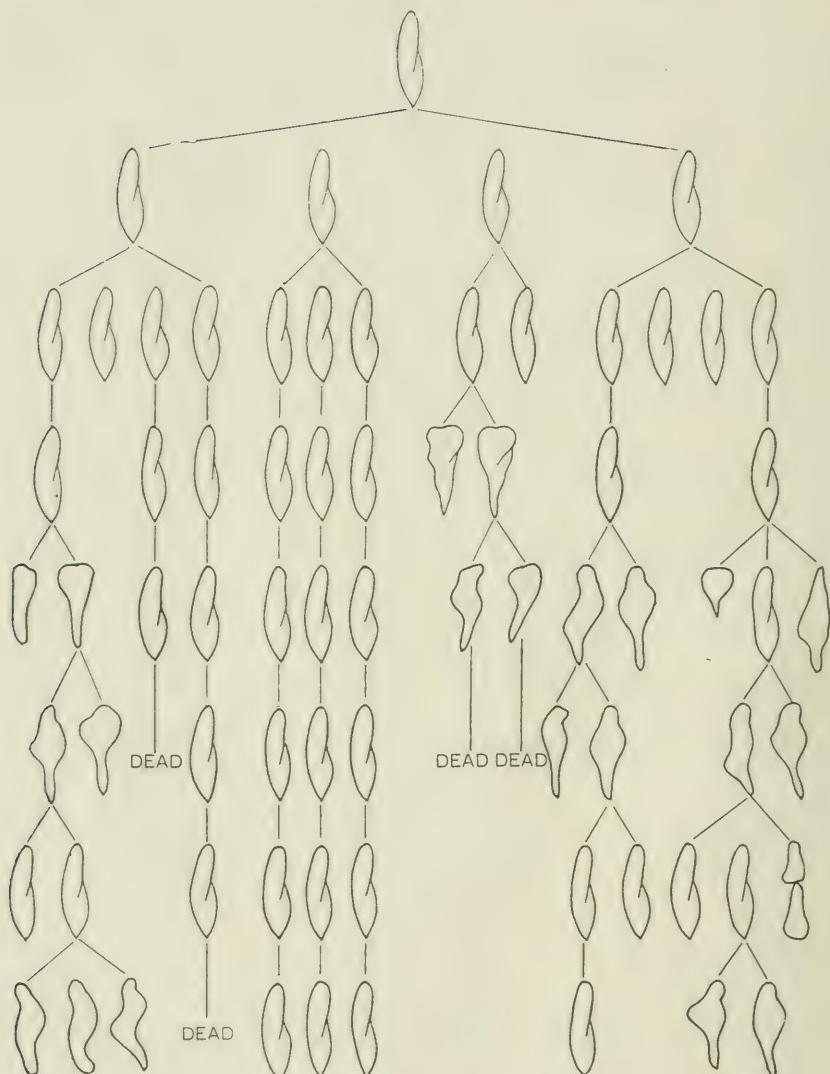


Fig. 12 Extract from the pedigree of Race A which includes the origin of the six normal lines produced by this race. In the abnormal lines only a few of the animals in each generation are shown; in the normal lines, only one in each generation.

continuous, and gave rise to 16 generations. Two normals and 18 abnormals appeared in the observed cultures of this line. The proportion of abnormality (79 per cent) shown by all these lines taken together is much higher than that shown by the race as a whole (56 per cent). Figures 13 and 14 give part of the pedigrees of three of these lines.

TABLE II

*Data from the 24 lines of Race A in which the selection of abnormals was continuous*

SELECTION CONTINUOUS					LINES ENTIRELY ABNORMAL		
Days	Generations	Normals	Abnormals	Per cent abnormal	Days	Generations	Individuals
16	9	4	13	76	10	6	10
18	6	3	6	67	12	3	3
8	2	1	2	67	6	2	2
6	1	0	1	100	6	1	1
8	1	1	1	50	6	1	1
6	4	2	4	67	4	2	3
6	2	0	2	100	8	1	2
18	6	8	8	50	10	1	1
36	16	2	18	90	28	12	14
20	3	3	3	50	12	1	1
16	5	5	6	55	8	2	2
22	11	4	11	73	8	3	3
24	8	6	8	57	12	2	2
20	9	4	8	67	8	2	2
22	8	3	6	67	12	2	2
20	6	0	6	100	20	6	6
24	5	0	5	100	24	5	5
8	4	1	4	80	6	3	3
12	4	1	4	80	10	3	3
14	4	2	4	67	10	2	2
12	3	0	3	100	12	3	3
10	2	0	2	100	10	2	2
6	8	8	10	56	2	1	1
14	8	22	12	35	10	5	5
Greatest							
36	16	22	18	100	28	12	14
Total.....		80	147	65			79

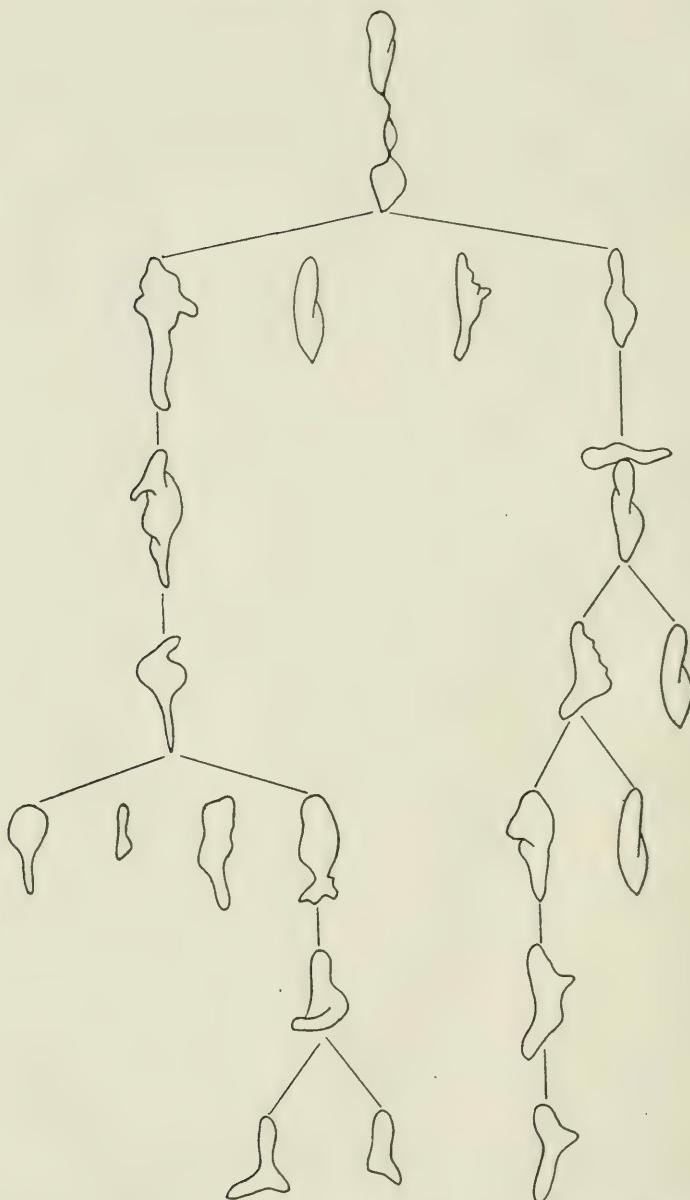


Fig. 13 Part of the pedigrees of two of the abnormal-selected lines of Race A.

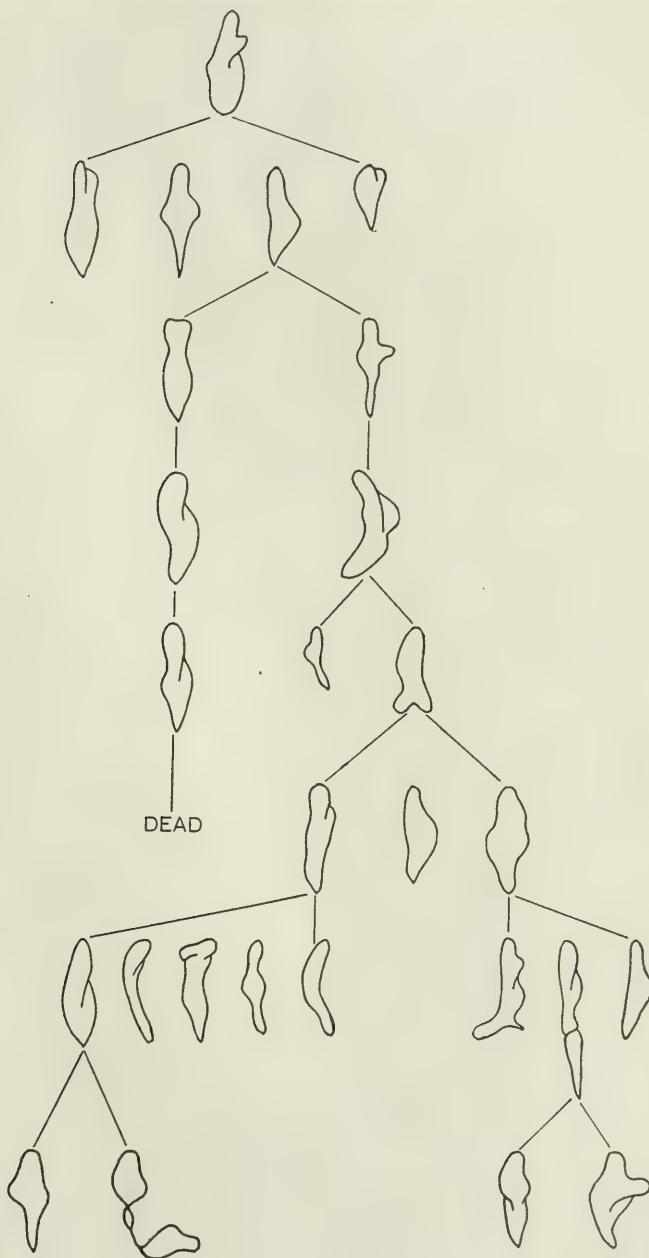


Fig. 14 Part of the pedigree of another abnormal-selected line of Race A.

We have therefore in race A a group of individuals all descended by vegetative reproduction from one animal, exconjugant 1b, which for 74 generations and 131 days constantly produced abnormalities in an average proportion of 56 per cent of all the individuals of the race. From this clone, having this heritable character, abnormality, were obtained through the action of selection, two diverse groups of individuals. One group had a constant proportion of abnormality, on the average, of 70 per cent; the other with almost total normality, the abnormality having been reduced to an average of 1 per cent; all of the lines were entirely normal for some time before they died out. In this clone therefore we have an inheritance of a variation, abnormality; and also we have permanent changes in this heritable variation, brought about by the action of selection.

TABLE 12  
*Data from fourteen groups of Race B*

GROUP	LENGTH OF LIFE IN DAYS	NUMBER OF GENERATIONS	NUMBER OF NORMALS	NUMBER OF ABNORMALS	PER CENT ABNORMALS
B1.....	81	47	43	73	63
B2.....	81	67	107	80	43
B3.....	81	73	178	144	45
B4.....	105	83	292	338	54
B5.....	75	61	60	94	61
B6.....	61	46	46	67	59
B7.....	71	66	114	177	61
B8.....	73	58	66	118	64
B9.....	89	64	73	110	60
B10.....	103	74	145	143	50
B11.....	67	46	40	137	77
B13.....	65	41	38	66	63
B14.....	69	53	87	145	62
B15.....	71	15	52	96	65
Greatest	105	83	292	338	77
Total.....			1371	1788	56

The history of race B shows some slight variations from that of race A. Its early history is shown in chart 7. It was en-

tirely normal until December 7, five days after conjugation. On that date two of the existing 27 normal individuals were chosen to carry on the race; one of these continued to produce only normals until it was discontinued six days later. By that time it had given rise to 53 normal individuals. This was the largest group of normals produced by race B.

CHART 7

(*n* = normal; *ab* = abnormal)

Early history of Race B and formation of the fifteen groups

December	3	5	7	9	11	13	15	17	Group
	1n—1	4n—4	27n—2	$\left\{ \begin{array}{l} 4n-1 \\ 2ab-2 \end{array} \right.$	$\left\{ \begin{array}{l} 4n-4 \\ 2ab-2 \end{array} \right.$	$\left\{ \begin{array}{l} 50n \\ 6n-6 \\ 2ab-2 \\ 7ab-7 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Discontinued} \\ 1ab-1 \\ 4ab-4 \\ 2ab-2 \\ 2ab-2 \end{array} \right.$	$\left\{ \begin{array}{l} \text{Dead} \\ 1ab \\ 1ab \\ 1n \\ 1n \\ 1ab \\ 1ab \\ 1n \\ 1n \\ 1n \\ 1ab \\ 1ab \\ 1ab \\ 1ab \end{array} \right.$	B1 B2 B3  B4 B5  B6 B7  B8 B9 B10 B11  B12 B13 B14 B15

The other normal kept on December 7 gave rise to all later individuals of race B, which were kept for 105 days, and 83 generations, and had an average proportion of abnormality of 56 per cent. The history of this race from this point on is exactly like that of race A. On December 23, 83 of the most abnormal lines were selected from the existing 314, and grouped (table 12).

Each one of the 83 lines was divided into two sub-lines, in one of which the most normal were selected, in the other the most abnormal. In six lines the continuous selection of normals was possible. Their history is given in chart 8, and table 13 gives the data from them. Two of these lines produced abnormals the last day they were kept, and a third was entirely normal for only four days before death; the selection has slightly decreased the proportion of abnormals produced, but has not eliminated the abnormal character. But the three other lines became entirely normal some time before death, as the table shows. Selection here has had a decided effect; has entirely eliminated the abnormal character.

## CHART 8

Genetic history of the six lines of Race B in which the selection of normals was continuous

(*n* = normal; *ab* = abnormal; *D* = died out)

Line	January						February					
	20	22	24	26	28	30	1	3	5	7	9	
			1ab	4ab	2ab							
B2	4n	8n	2n	4n	2n	4n	8n	2n	4n	4n	4n	D.
B3		8n	4n	8n	6n	8n	8n	2n	4n	3n	3n	D.
Line	December						January					
	25	27	29	31	2	4	6	8	10	12	14	16
			3ab	1ab	2ab							
B4b2	2n	1n	1n	1n	2n	1n	8n	2n	5n	2n	2n	4n
			2ab			1ab	3ab		3ab	1ab	2ab	
B4i1	2n	2n	2n	2n	2n	2n	1n	3n	1n	1n	2n	2n
	3ab	2ab	2ab	1ab	2ab	1ab	2ab	1ab	1ab	3ab	1ab	1ab
B7	1n	2n	2n	2n	2n	1n	1n	1n	2n	1n	1n	3n
	2ab			1ab								
B15	2n	2n	2n	2n	1n	1n		D.				

In nine lines the continuous selection of abnormals was possible. The history of three of them is given in chart 9 and table 14 gives the data from them. The proportion of abnormality in most of these lines has been somewhat raised by this selection over that of the race as a whole; but not to such an extent as in

race A. Pedigrees of two of these lines are given in figures 15 and 16.

TABLE 13

*Data from the six lines of Race B in which the selection of normals was continuous*

SELECTION CONTINUOUS					LINES ENTIRELY NORMAL		
Days	Generations	Normals	Abnormals	Per cent abnormal	Days	Generations	Individuals
20	20	33	7	17	10	11	18
18	22	43	0	00	18	22	43
28	21	30	6	17	20	17	29
26	13	15	12	44	4	4	5
30	13	16	20	56	0	0	0
12	4	5	3	38	0	0	0
Greatest							
30	22	43	20	56	20	22	43
Total.....		142	48	25			95

## CHART 9

Genetic history of three of the lines of Race B in which continuous selection of abnormals was made

(n = normal; ab = abnormal)

January														
Dec.	31	2	4	6	8	10	12	14	16	18	20	22	24	26
	1n		1n	1n	1n									
1ab	3ab	3ab	3ab	3ab	3ab	2ab	1ab	1ab	1ab	Dead				
2n					3n	3n	3n		2n	1n				
2ab	2ab	4ab	2ab	5ab	1ab	1ab	2ab	2ab	4ab	1ab	1ab	1ab	Dead	
	1n	3n		2n										
2ab	3ab	1ab	2ab	6ab	4ab	4ab	1ab	2ab	4ab	1ab	Dead			

We have therefore in race B a group of individuals constituting a clone, which after the fifth generation and the production of 32 individuals, gave rise to two diverse groups, with different hereditary characteristics. One group was entirely normal; the other group showed a large and constant proportion of abnormals. Later from this abnormal group, by continued selection in opposite directions, lines were isolated which showed

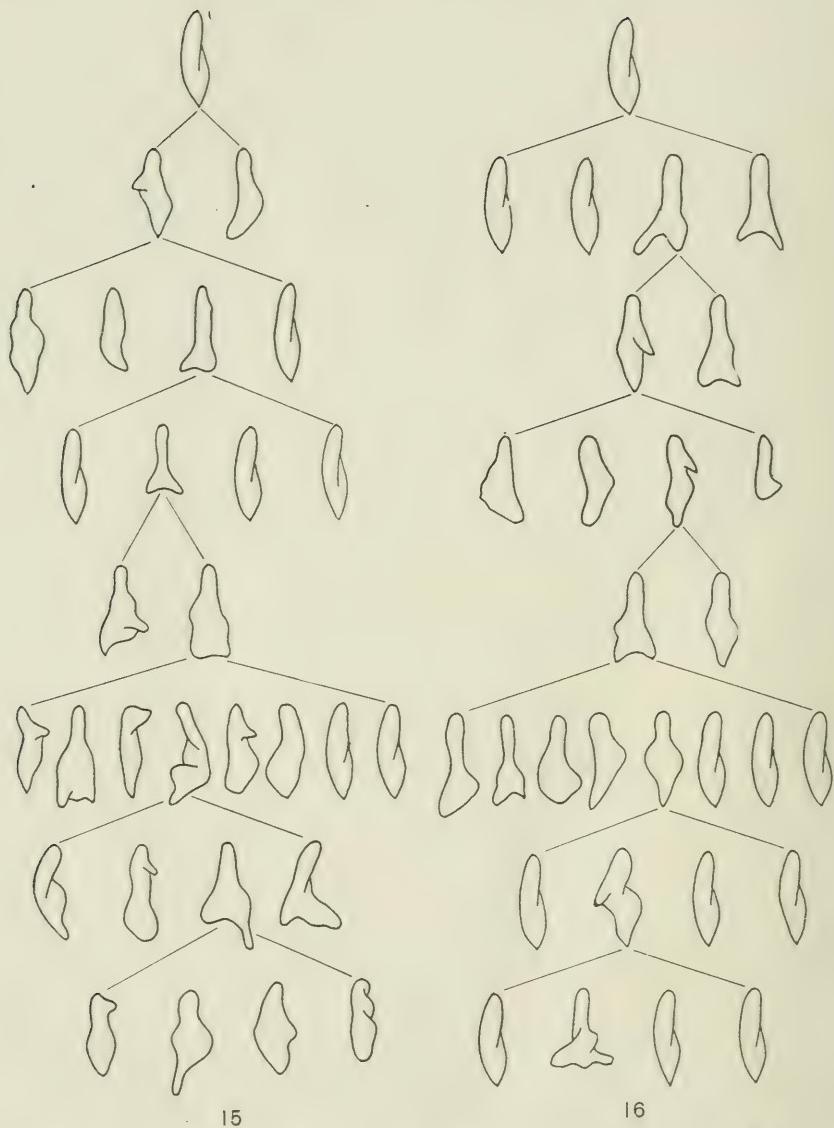


Fig. 15 Part of the pedigree of an abnormal-selected line of Race B.

Fig. 16 Part of the pedigree of another abnormal-selected line of Race B.

TABLE 14

*Data from the nine lines of Race B in which the selection of abnormals was continuous*

SELECTION CONTINUOUS					LINES ENTIRELY ABNORMAL		
Days	Generations	Normals	Abnormals	Per cent abnormal	Days	Generations	Individuals
20	9	2	20	83	8	5	5
18	8	4	11	73	10	2	2
18	6	5	8	62	8	2	2
24	11	14	16	53	2	1	1
18	9	1	10	91	14	8	8
22	13	6	20	77	12	8	11
22	10	13	15	54	2	2	3
24	13	11	15	58	2	1	1
14	5	2	6	75	12	5	5

hereditary differences in degree of abnormality. One set of lines showed a very low degree, three lines becoming entirely normal. The other set showed a very high degree of abnormality, one line having 90 per cent of abnormals.

We have then in race B the inheritance of a variation within the clone; and a splitting up of the clone, both with and without selection, into hereditarily diverse groups.

The six races of Experiment 3 underwent a most strict selection of normals throughout their history; and in every case this eventually brought about a change in the inheritance of the abnormality. In each race all of the individuals arising from the exconjugant were kept for the first six days; thereafter the abnormals were discarded and only normals kept, as far as possible. Abnormals were kept only when no normals were present with which to carry on the race. In all these six races this procedure had much the same effect. Some of their lines were not changed at all; they continued to produce abnormals from the normal cells selected. But other lines became entirely normal. In every case there was inheritance of the variations which had arisen within the clone. Figures 17, 18, 19, and 20 give pedigrees of four of these races, 56a and b, and 59a and b. All six races are very similar in their history. On January 17 none of the six had completed their first division; all had formed

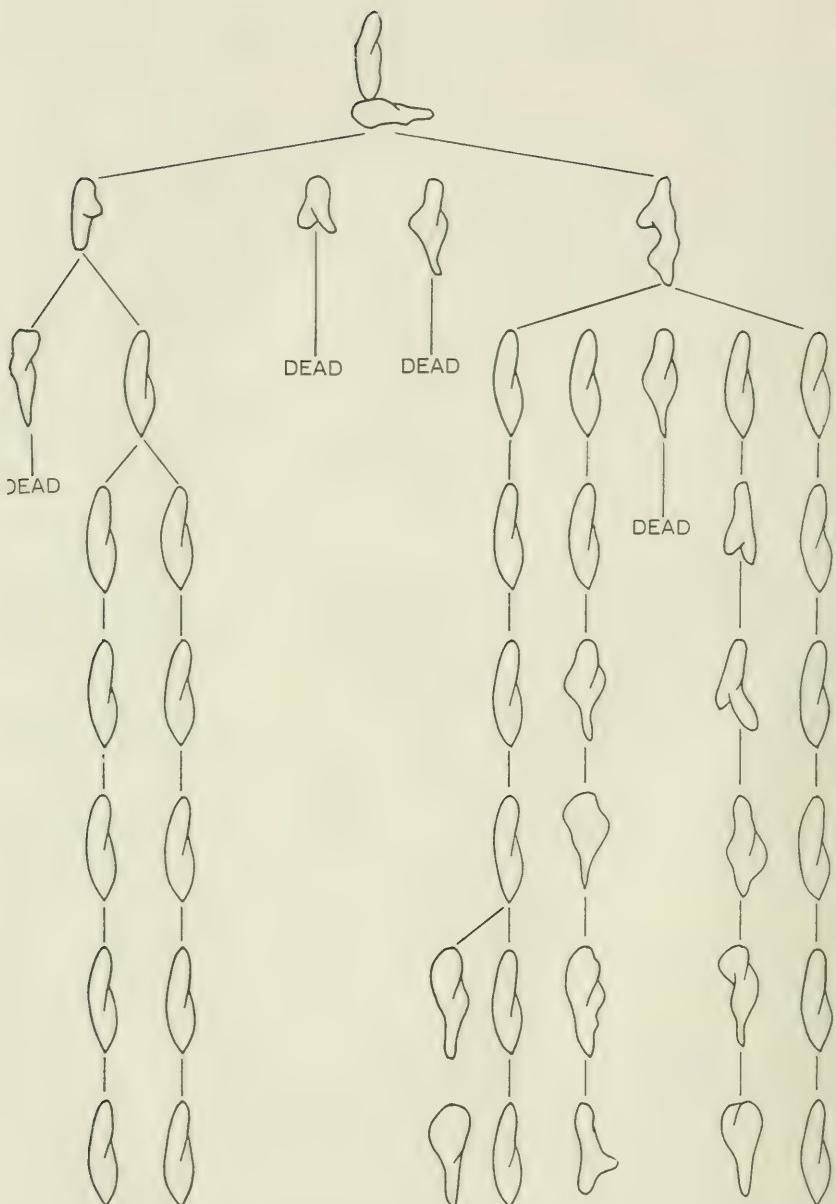


Fig. 17 The first part of the pedigree of 56a, showing the origin of four of the normal lines. The other four normal lines were derived much later from one of the abnormal lines shown here. In this figure and in the succeeding ones, in each generation only those animals are shown which were selected to carry on the lines.

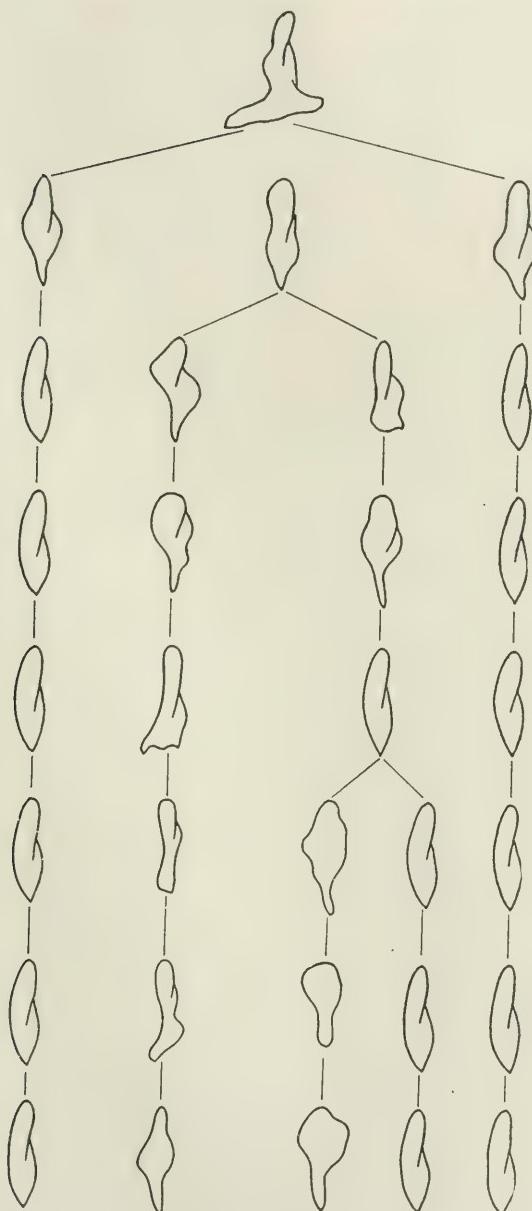


Fig. 18 First part of the pedigree of 56b, showing the origin of three of the normal lines. The other eight normal lines were derived much later from the abnormal lines shown here.

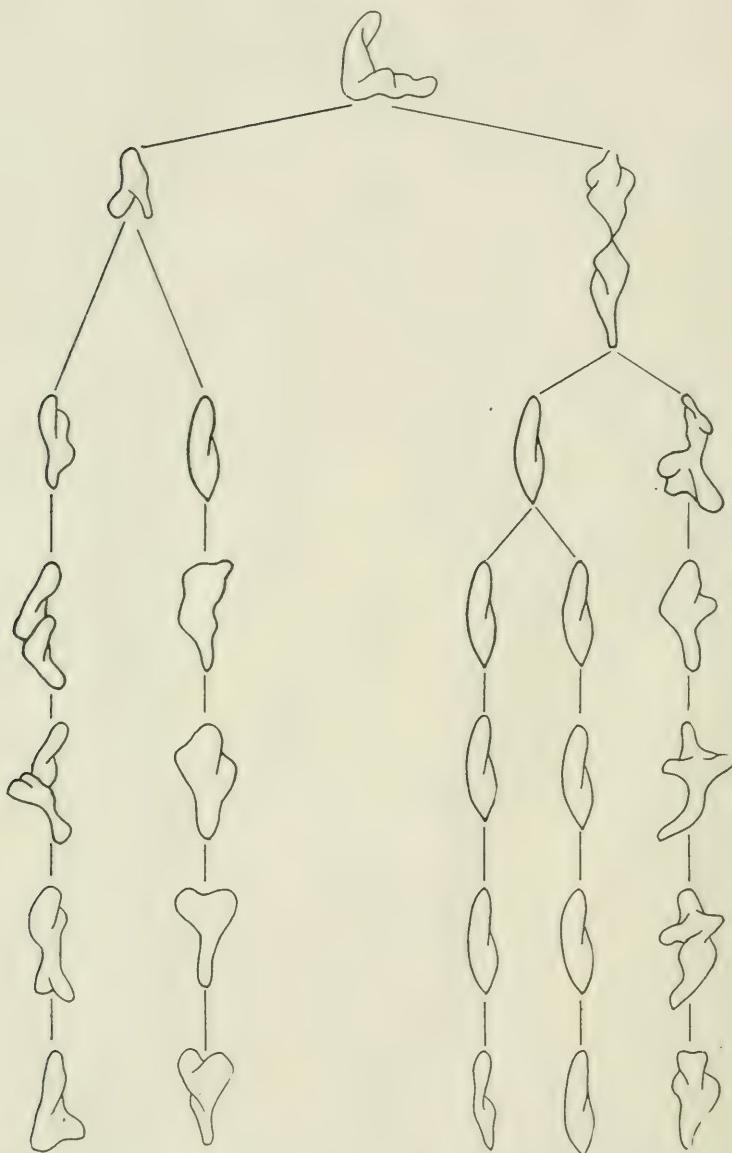


Fig. 19 The first part of the pedigree of 59a, showing the origin of one of the normal lines. The other normal line was derived 32 days later from one of the abnormal lines shown here.

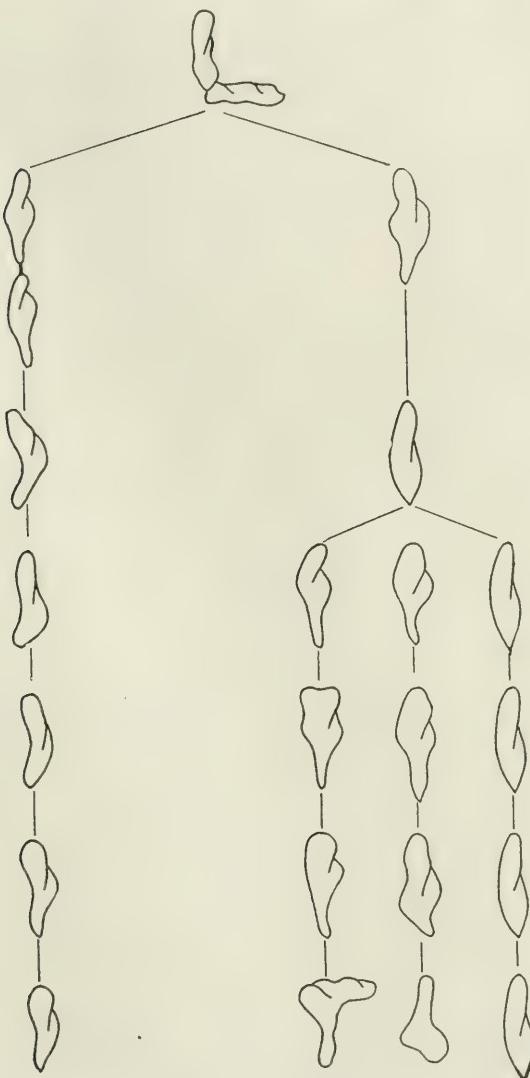


Fig. 20 The first part of the pedigree of 59b, showing the origin of one of the normal lines. The other eleven normal lines were derived much later from the abnormal lines shown in this figure.

double monsters of the L variety. On January 19, exconjugant 56a (fig. 17) had divided to form four abnormalities; two of these died without dividing further. One of the other two produced

one abnormal which died before dividing, and one normal which gave rise to two entirely normal lines which were kept for ten days and eleven generations. They comprised 115 observed individuals, all normal. The other abnormal present on January 19 divided to form one abnormal (which died before further division) and eleven normals. Nine of these were kept. Six of them gave rise to lines which remained entirely normal; three produced abnormal lines. Two of these abnormal lines remained abnormal throughout their history, never responding positively to the normal selection they underwent. From the other abnormal line five entirely normal lines were split off, kept for a number of generations varying from 6 to 24. Three of these normal lines were kept until April 1; their eight abnormal sister lines were also kept until April 1, 77 days after conjugation.

Since all six lines had very similar histories they will not be given in detail. The main facts as to the production of the normal lines can be gathered from the pedigrees (pages 436 to 439) and the table (pages 421-422).

Thus on the whole the work on selection shows that with relation to these abnormal characteristics heritable variations are in many races occurring during vegetative reproduction. By selection the effect of these variations may be accumulated, so that while one part of the race remains abnormal, or even increases the proportion of abnormal individuals, another part greatly decreases the proportion of abnormality, or becomes entirely normal. The general bearing of these results will be discussed after the rest of the facts have been brought out.

#### *Relation to biparental inheritance*

Does conjugation tend to produce similarity in respect to abnormality or normality between the progeny of the two individuals that conjugate? That is, if the descendants of one of the two members of a given pair are abnormal, is there a tendency for the descendants of the other member to be abnormal also? If a given stock is abnormal, is the stock derived from the mate of its progenitor likely to be abnormal also? Jennings and Lashley ('13, '13a) have shown that there is such a tend-

ency to resemblance between the stocks derived from the two members of a pair, in respect to fission-rate, death-rate, and size.

The question with relation to abnormalities may be attacked by the same methods employed by Jennings and Lashley. The problem presents itself as follows. From a certain number  $m$  of exconjugants (forming  $m/2$  pairs) a certain number  $n$  of abnormal races are produced. In some cases both races derived from a pair are abnormal; in other cases only one; in others, neither. Is the number of cases where both races are abnormal greater than would be expected if the pairing had no relation to the distribution of the abnormalities? If so, this is evidence that the pairing tends to make the two races alike in this respect.

Jennings and Lashley ('13) give a formula for determining the most probable number of pairs that will be found to be alike in respect to any such character if the pairing has nothing to do with the distribution. This formula is:

$$k = \frac{(n + 1)(n/2 + 1)}{m + 3}$$

in which the nearest integer below  $k$  is the most probable number of pairs in which the two members will be found alike in the character in question;  $n$  is the number of cases (lines) in which this character occurs, while  $m$  is the total number of cases (lines of descent from exconjugants in this case). (This formula holds absolutely only when  $n$  is even, but by obtaining the result for the two even numbers above and below the actual number, if the latter is odd, the difficulty may be avoided.)

In examining this matter for our case with respect to normality and abnormality, 17 of the 131 pairs of the first experiment must be omitted from consideration, since in these the characteristics of one or both members is unknown. This leaves 114 pairs to be dealt with, giving 228 lines of descent. In the second experiment 3 of the 100 pairs must be omitted from consideration, leaving 97 pairs to be dealt with, giving 194 lines of descent. In the third experiment there are 14 pairs to be considered, giving 28 lines of descent. This makes 450 lines of descent all together.

Of these 225 pairs, in the first experiment both members in 48 gave normal lines, both members in 26 gave abnormal lines, while in 40 the two members gave lines diverse in this respect. The total number of normal individuals was therefore 136, of abnormal individuals 92. In the second experiment, both members in 7 gave normal lines, both members in 69 gave abnormal lines, while in 21 the two members gave lines diverse in this respect. The total number of normal individuals of experiment 2 was therefore 35; of abnormal individuals 159. In Experiment 3, both members in 8 pairs gave normal lines, and both members in 6 gave abnormal lines; pairing was perfect. The total number of normal lines of Experiment 3 was therefore 16; of abnormal lines, 12.

How many pairs with both lines abnormal should we expect to find in these cases if pairing does not affect the distribution of abnormalities? In the first experiment  $n$  is 92, while  $m$  is 228. Applying the formula we find that the expected number of pairs with both members abnormal is 18; the actual number is 28. In the second experiment  $n$  is 159 while  $m$  is 194. By the formula the expected number of pairs with both members abnormal is 66, while the actual number is 69. In the third experiment  $n$  is 12 while  $m$  is 28. The expected number of pairs is 2, the actual 6. In the same way the expected number of pairs with both members normal is in the first experiment 40, while the actual number is 48; in the second experiment the expected number is 3 the actual 7; in the third, the expected number is 4 the actual 8.

It is therefore clear that in all three experiments the number of like members of pairs is greater than would be the case if the pairing had no effect on the distribution of the abnormalities. *Conjugation tends to cause the descendants of the two members of pairs to become alike in respect to abnormality and normality.*

Some of the exconjugants never divided after separation. The same question may be examined with respect to them. Do the two members of a pair tend to have the same fate in this respect? There were 72 exconjugants that never divided, out of the 228 of the first experiment, and 27 out of the 194 of the second

experiment. In the first experiment both members in 19 pairs were affected; in the second experiment, both members in 8 pairs. Applying the formula (first experiment,  $n = 72, m = 228$ ; second experiment,  $n = 27, m = 194$ ) we find that if pairing has no effect on the matter, the most probable number of cases for both members in the first experiment is 11; in the second, 2. Similarly there are in the first experiment 61 pairs in which both members divided, while the most probable number is 53. In the second experiment the actual number is 79, the most probable is 72. Again we find that conjugation increases the resemblance of members of pairs in this respect.

It will be of interest also to give the relation of pairing to the date on which the lines finally died out. This is known for both members of but 51 pairs of the first experiment, and 41 of the second. The numbers in the third experiment are too small to be considered. In some cases the descendants of both members of a pair completely died out on the same date; in other cases the descendants of one member lived longer than those of the other.

The main facts are these. In the first experiment, on December 5, twenty lines were found to have died out; these included both members of 8 pairs. The probable number of pairs if conjugation does not affect the date of death is but 2. On December 7, 53 individual lines ended, including both members of 20 pairs. The most probable number of pairs would be but 14. On later dates the numbers are too small to be significant. In the second experiment on December 6, 31 lines were found to have died out; these included both members in 7 pairs. The probable number of pairs if pairing does not affect the date of death is but 6. On all the other dates the numbers were so small that they are not worth considering. Conjugation thus tends to make descendants of the two members of a pair alike in their length of life (in their 'vitality').

All together, the evidence shows that conjugation induces resemblances in the two members of a pair in respect to all the characters examined: in the tendency to fail to reproduce after conjugation; in the abnormalities produced by their offspring; and in the length of life of the stocks produced.

## VII. SUMMARY AND DISCUSSION OF RESULTS

Among the progeny of a large proportion (from 36 to 81 per cent in the different experiments) of exconjugants of *Paramecium caudatum*, abnormalities appear frequently and constantly.

These abnormalities show themselves to be hereditary in the following respects:

1. Lines derived from different exconjugants differ in respect to them: some lines show no abnormalities; others show a small proportion of abnormal individuals; others large proportions up to cases in which abnormality is almost or quite universal. The tendency to abnormality is transmitted in fission; definite proportions of abnormality being characteristic of particular lines. In one case there was inheritance of a specific type of abnormality carried through 303 generations (Race C).

2. The diversities in abnormality occurring within a single line of descent (derived from a single exconjugant) are in some lines not hereditary, so far as can be determined by long continued selection. In a very large proportion of the races in which the abnormals were regularly discarded and only normals retained to carry on the race, the abnormal character persistently reappeared, the selected normals producing abnormal progeny. In all the abnormal races there is a wide variation in degree of abnormality of the individual, from those perfectly normal to the monsters so deformed that they would never be recognized as paramecia if their history were not known. Yet, as stated above, in most cases the progeny of all these variations were alike, the daughter cells of normal individuals being often just as abnormal, or even more so, than the daughter cells of monsters. This of course agrees with the conditions found in most of the studies on inheritance in 'pure lines' or clones: the diversities within the lines are not inherited.

3. But in other lines diversities within the line showed themselves to be heritable, so that selection gave very different results from those usually obtained in pure line work. By selection, single lines, derived by fission from a single parent, were divided into two or more races differing hereditarily. This was success-

fully accomplished in twenty-five races; from each of these were isolated two sorts of lines, one quite normal, the other continually producing abnormalities—the two cultivated side by side.

Calkins and Gregory ('13) have in some cases obtained four diverse races from the four primary daughter cells, or 'quadrants' of an exconjugant—these being the four individuals that receive the four macro-nuclei produced before fission occurs. It is to be noted that our selection resulting in the isolation of lines differing hereditarily in abnormality has often been brought about much later in the series of generations, so that the differentiation has occurred within the compass of a single 'quadrant,' or indeed within a much narrower fraction of the descent. In several cases differentiation through selection did not begin till after several weeks had passed with production of a great number of generations. Thus the results of selection in the present case cannot be interpreted as due to a primary difference in the four original macronuclei produced during conjugation. Selection is effective when begun with progeny of a single individual that has appeared many generations after conjugation.

4. In a race of *Paramecium* which upon extended examination shows no hereditary abnormalities, conjugation results in the appearance of many lines which are hereditarily abnormal, others which are normal throughout (Experiment 2).

This is of course an example of what Jennings ('13) has described as the 'production of variation by conjugation.' It appears to fully meet the desire of Dobell ('14, p. 172) for "convincing evidence of a concrete instance in which from a known race—constant in a certain character—a new race—permanently diverse in this character—has arisen as a result of conjugation." For in Experiment 2, from a clone with a constant character of normality (as shown by the progeny of the 54 split pairs of this clone) 101 races which were permanently diverse from the original one, being hereditarily abnormal, arose as a result of conjugation.

5. In the diverse lines descended from the different exconjugants of a conjugating culture, the two lines descended from the two individuals that have conjugated together tend to be

alike in respect to normality or abnormality. That is, if the progeny of the exconjugant *a* are abnormal, the progeny of its mate *b* are more frequently abnormal than would be the case if the distribution of abnormal races were not affected by conjugation. This is an example of what Jennings and Lashley ('13) have called biparental inheritance as a result of conjugation. As these authors point out and as Dobell ('14) has recently emphasized, this does not mean that the characters of the progeny of the two exconjugants are known to resemble the characters which the two parents had before they conjugated. It means merely that the characteristics of the progeny of *a* are not determined by the nature of *a* alone, but partly also by the fact that *a* has conjugated with *b*. Just what the resulting similarity of the progeny of *a* and *b* should be called is of little importance, as compared with a clear grasp of the facts in the case, yet it is perhaps worth while to point out that similar relations often appear in what is called inheritance in higher organisms. Two heterozygotic parents frequently produce progeny which differ from both of them, yet what the progeny shall be is determined by the constitution of both of the parents.

6. We are dealing here with characters that are called abnormal. What is the bearing of this on any conclusions drawn from this study? Can we learn anything worth while from the study of abnormal characters?

What we can learn from abnormalities, and what the relation of their behavior is to that of other characters can be determined only by investigation; the present paper is offered as a contribution toward answering such questions. But certain general principles appear worthy of consideration. What is the real status of the conception of abnormality? Does it mean anything more than that the condition so characterized is not the one usually found? It certainly does not mean that the condition is one not subject to law of any sort. If we characterize the course of inheritance of these characters as abnormal, we can mean no more than that they do not follow the usual course. But the course they do follow is one actually occurring in animals, and therefore one not inconsistent with the nature

of organic matter or the constitution of organisms. It is perfectly possible for organisms to exist in which hereditary variations occur during non-sexual reproduction, and in which these variations can be accumulated through selection; for in the characters here studied this does occur. No one therefore can say *a priori* what characters must descend in this manner, what in some other manner.

It is then an open question whether a similar scheme of descent may not be followed by other characters, either in this same organism, or in other organisms. Jollos ('14) has raised the question whether all the hereditary variations shown by Jennings to follow upon conjugation may not be of the nature of abnormalities. It is entirely possible that all show the same scheme of descent as the 'abnormalities' considered in this paper; but if so then the 'abnormal' cannot be characterized as the unusual. In the same way it is possible that the hereditary differences among the progeny of single exconjugants observed by Calkins and Gregory ('13) may be of the same nature as these abnormalities. It is quite possible that lines might be 'abnormal' in ways evident only through study of the fission rate, or the like. But with such a state of the case the distinction between abnormal and normal becomes evanescent.

The facts are, that in *Paramecium* as a result of conjugation, there appear lines or races that are hereditarily diverse; and that within some of these lines hereditary diversities likewise appear even during asexual multiplication. Whether we can maintain some special category such as 'abnormality' for all these cases, can be discussed; but this does not do away with the facts as to their existence and the scheme of their descent from generation to generation.

It is of interest to compare the genetics of these abnormalities in *Paramecium* with abnormalities in other organisms. Almost all the characters studied by Morgan and his associates in *Drosophila*, on which have been reached results of such immense importance for the entire theory of genetics, may be characterized as abnormalities. A large proportion of them show, as is well known, typical Mendelian inheritance. Others show a

more irregular course, resembling in this respect those of *Paramecium*. Such a character is the 'beaded wing,' recently studied by Dexter ('14). Lutz ('11) had previously investigated an abnormal wing character in *Drosophila*, with results that are still more similar to the conditions shown in *Paramecium*. Lutz found that certain abnormalities of venation were heritable, but, as in *Paramecium*, the inheritance was not precise; the abnormalities of parent and offspring might be diverse, but the tendency to produce some sort of abnormality of venation was inherited. Furthermore the proportion of individuals abnormal, and the degree of abnormality, were modifiable by selection, apparently through fine gradations. Lutz is of course working with bisexual reproduction, which greatly complicates the interpretation of the matter. Yet selection continued to be effective after ten generations of inbreeding, at which time it would be expected that a homozygotic strain would have been reached, if the character were dependent on typical Mendelian units.

In many other cases abnormalities have been shown to follow aberrant types of heredity; to attempt a general review of the matter here would lead too far.

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# HERITABLE VARIATIONS AND THE RESULTS OF SELECTION IN THE FISSION RATE OF STYLONYCHIA PUSTULATA

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SEVENTEEN FIGURES

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## INTRODUCTION

Organisms multiplying without admixture of two parents that differ in hereditary constitution have been found remarkably constant in their inherited characteristics. Most recent work agrees that in such uniparental reproduction inherited variations occur rarely or not at all, and that selection has practically no effect in altering racial characteristics. These results have given origin to the concept of the Genotype (Johannsen), as a designation for the permanent heritable constitution of the race. In view of the importance of these relations for the problem of the method of evolution, much further study of this matter is required. The present paper deals with the inheritance of varia-

tions and the effect of selection in the case of a most delicately poised and readily modifiable physiological character, the rate of fission of an infusorian multiplying without conjugation.

Most studies of the fission rate in infusoria have consisted in observations on the rate in given species, for descriptive purposes; or in examinations of the direct effect of changed physical and chemical conditions; or in the study of the effect of conjugation on the rate of multiplication. Review of these studies is not demanded here.

A certain number of observations have been made which bear on the inheritance of the fission rate and on the question whether there are differences among the progeny of a single individual in respect to these. The great papers of Maupas ('88 and '89) contain some of the most important of these. Maupas made extensive studies of the effects of temperature and of other conditions on the fission rate in many species of infusoria; among the rest in *Styloynchia pustulata*, the subject of the present study. Maupas became convinced, as a result of his studies, that under given conditions the rate of fission is uniform in all the progeny of a given individual; that inherited variations in the rate do not occur in such a 'pure race' or to use the modern term, within a single clone. On this matter the conclusions of Maupas are in complete harmony with those of the 'pure line' workers and upholders of the constancy of the genotype, in more recent times. He gives detailed observations on the matter for a number of species, and resumes his results as follows:

In all my cultures I have always seen all the normal descendants of the same ancestor, grow and multiply with the most perfect uniformity. I have become convinced of the integral transmission of the faculty of development from one generation to another, and the most complete physiological equivalence must exist among all the normal individuals, produced in the successive generations ('88, p. 203).

In long and numerous experiments on fifteen to twenty species, I have never observed anything which permits belief in the existence of morphological and physiological differences, not merely between the two products of a given fission, but even among all those which have descended from such a fission by regular and continuous generations ('88, p. 176).

The paper of Jennings on the Effect of Conjugation in Paramecium ('13) likewise deals to a certain extent with this matter. In a wild population many strains differing in rate of fission (under the same conditions) were found to occur. Furthermore, it was demonstrated that even in a population derived by fission from a single individual (that is, in a 'pure strain'), conjugation produced inherited differences in the fission rate, so that after conjugation there were present strains showing constant differences in these respects.

On the other hand, if no conjugation has occurred among the progeny of a single individual, the fission rate was found to be nearly or quite uniform. Jennings sums up as follows:

It is found (1) that differences in rate of fission among those that have not conjugated since they were derived from a single parent are not inherited (unless possibly certain differences of a minimal character are to be excepted; differences of an order of magnitude far below those with which we are dealing); (2) that conjugation among the members of such a pure race does result in differentiations that are inherited ('13, p. 366).

The paper of Calkins and Gregory ('13) on the other hand sets forth that there are in many cases differences in the fission rate among the four sets of progeny resulting from the first two divisions of an individual that has just conjugated.

Other papers bearing less directly on this matter will be taken up in the discussion of the results of the present work.

#### *The specific problem*

When a single infusorian divides, often one of its two progeny again divides before the other does. In successive generations this same thing may be repeated. Thus, as shown in figure 1, one may have among the progeny of a single individual at a given moment, animals that are the products of four and others that are the products of two fissions. Hence there are differences of fission rate among the descendants of a single individual—differences that afford the opportunity of selection with a frequency that made it appear worth while to determine whether these differences are inherited and whether slow lines

and fast lines can, by selection, be isolated among the descendants of a single parent. I have carried out this work for *Styloynchia pustulata*. The animals were kept isolated and transferred daily to fresh culture medium; for the fast lines the individuals that divide first are uniformly selected; for the set to be developed into lines having a slow rate of fission the individuals that divide last are taken.

No attempts have ever been made heretofore to test the effect on the fission rate of selection among the progeny of a single individual. It appeared possible, though hardly probable, that such a physiological character might give results differing from those obtained from studies of the mainly structural characters hitherto examined. The work was undertaken at the suggestion of Prof. H. S. Jennings, to whom my sincere thanks are due for assistance throughout the work.

The fundamental questions for examination are then as follows: Can we, with respect to the character examined, get from a single genotype by selection two genotypes that differ characteristically from each other under identical conditions; and that retain these differences from generation to generation? Is selection of small variations, such as appear within the pure strain or clone an effective evolutionary procedure?

#### TECHNIQUE

In any investigation of a physiological character which is so delicately responsive to all environmental changes as is the fission rate of infusoria, the statement of Calkins ('02, p. 141)—“A correct method is the sine qua non of successful experiments with Protozoa;”—applies with peculiar force. In order that results may be of any value conditions must be uniform throughout.

Jennings ('13) has pointed out that to secure this uniformity the bacterial content must not vary. In addition to uniformity the technique used in work on the fission rate must insure the experimenter against the introduction of a ‘wild’ individual into the cultures and against the contamination of the lines inter se. These results have been secured by the adoption of the follow-

ing method, which is a modification of that described by Jennings ('13):

As culture medium,  $\frac{1}{16}$  of 1 per cent Horlick's malted milk was employed, a fresh supply being made daily. This is the medium adopted by Miss Peebles ('12). One gram of the malted milk powder was dissolved in a 100 cc. graduate in a few cc. of boiling spring water; this was then diluted to 100 cc. with more of the boiling spring water. Six and one-quarter cc. of this 1 per cent solution were next diluted to 100 cc. with boiling spring water and this  $\frac{1}{16}$  per cent solution was filtered and cooled.

The animals were cultivated on ground glass slides having each two circular depressions capable of holding four or five drops of liquid apiece. These were kept in moist chambers. Three drops of culture medium were used in each depression and no cover-glasses were employed. The 'fast' lines were kept in the left concavities and the corresponding slow lines in the right concavities of the same slides, so that conditions were uniform for the two sets.

Uniformity of bacterial content in the culture medium was secured by washing the animals in fresh culture medium before transferring to a new slide. The animals were allowed to swim about for a time in the fresh medium, in order to wash themselves largely free of bacteria; they were then transferred to the definitive slide, in new fluid. The pipette used in transferring the individuals was invariably sterilized in boiling water after each transfer, thus absolutely preventing the unintentional introduction of any individual which might cling to the pipette; there was thus no possibility of admixture of the 'fast' and 'slow' sets. The slides were labeled in lead pencil; the number of fissions and selections at each examination were likewise recorded on them, to be later transferred to permanent records. The individual lines were designated in accordance with the plan set forth by Jennings ('13).

Each concavity contained characteristically two parent individuals, the products of a single fission. The slides were examined daily or oftener. When one of the two individuals was

found to be divided, while the other was not, a selection was made (fig. 1). If the line is under selection for the production of a rapid rate of fission, the two progeny of the individual that has divided are transferred to the new slide, while the undivided individual is rejected. In selection for a slow rate of fission, on the other hand, the individual not yet divided is selected for

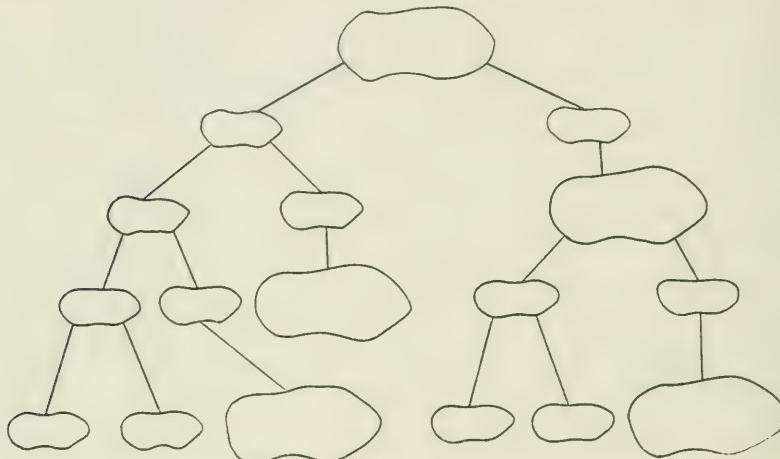


Fig. 1 Diagram of successive fissions among the progeny of a single individual, illustrating the variations in fission rate which were the subject of selection in the present work. The left side of the figure traces a series of 'fast' selections, the right side a series of 'slow' selections, showing that at a given moment we may have, among the progeny of a single parent individuals of the fourth and of the second generation.

propagating the stock. This process is continued: a 'selection' is made and counted whenever one of the two parents is found to have thus divided before the other.

#### EXPERIMENTS TO TEST THE EFFECT OF SELECTION ON THE FISSION RATE WITHIN A SINGLE CLONE

##### *1. The first series of experiments*

*Experiment 1, part 1.* Direct selection in opposite directions, November 3 to December 3, 1913.

On November 3, sixty animals of the sixth generation from a single individual which had been isolated from a 'wild' labora-

tory culture of *Styloonychia pustulata* were isolated on ground glass slides, one animal to each concavity, thus dividing the sixty animals into two groups of thirty each; one group to be selected for a fast fission rate ('plus selection'), the other for a slow one ('minus selection'). For twenty-one days these thirty 'fast' and thirty 'slow' lines were propagated with frequent selection as described above. On the twenty-second day the fast and the slow lines were duplicated (by division) and on the twenty-third day the first forty of the resulting sixty fast lines and the corresponding slow lines were all duplicated so that now we had one hundred fast lines and one hundred slow lines, plus and minus selection continuing with all these. These were thus propagated until the end of the third ten-day period. The actual number of fissions per line and the actual number of selections that were made during the three ten-day periods are shown for the first thirty lines of each set in juxtaposition in table 1.

When the differences per ten days of the sixty lines are averaged it shows that on the average the 'fast' lines have produced 2.03 generations more than the average for the 'slow' lines during the first ten-day period, 3.57 generations more in the second ten-day period and 2.40 generations more in the third ten-day period. Further, the fast lines have each averaged 2.67 generations more per ten days, during the whole thirty days, than the slow lines. In other words, this table shows that on the average each of these thirty fast lines has produced 0.267 generation more per day during this thirty-day period than has each slow line. Figure 2 is the curve of the difference between the daily averages of the two sets.

It is clear that the direct effect of the selections made would be to produce a difference in favor of the 'fast' lines as long as plus and minus selection continues, even though the differences were not hereditary and were due purely to accidental causes. Our next test is therefore to determine whether any hereditary result has been produced; to do this, selection must cease, and we must determine whether the differences in rate still continue. The most obvious method would be to stop selecting and pick

TABLE I

*Experiment 1, Part 1: Number of fissions (generations) and number of selections per line per ten-day period, for the first thirty lines of both the fast and slow sets while opposite selection was in progress; with the excess in favor of the fast set. (Differences given the minus sign show an excess of the slow set)*

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE PER LINE
First 10-day Period:																																
Fast:																																
Selections.....	3	2	2	3	2	4	3	3	4	5	3	2	3	3	4	1	2	4	3	4	4	4	4	7	5	5	5	5	3	2	19.76	
Generations.....	19	20	18	19	22	22	22	20	17	19	17	19	20	19	19	19	19	19	20	23	19	20	21	21	20	20	23	16	593	19.76		
Slow:																																
Selections.....	2	4	1	3	2	5	2	2	4	7	7	4	3	4	6	4	4	6	4	3	5	4	3	2	5	6	3	4	7	4	17.73	
Generations.....	18	17	18	16	18	18	16	19	18	17	15	19	16	15	17	19	15	19	18	16	18	17	20	18	19	18	19	21	532	17.73		
Excess in favor of 'fast'.....	1	3	0	3	1	4	6	3	2	0	5	0	1	4	3	0	4	0	1	4	5	2	0	3	1	2	2	2	4	-5	61	2.03
Second 10-day Period:																																
Fast:																																
Selections.....	3	6	4	5	5	5	5	5	4	5	4	4	6	5	6	5	7	5	5	5	4	5	4	5	6	6	5	5	6	6	19.00	
Generations.....	21	17	17	18	18	21	18	21	18	21	19	20	20	18	18	17	20	17	16	17	18	19	21	21	19	18	19	23	18	570		
Slow:																																
Selections.....	5	5	2	7	4	4	4	3	6	5	7	5	6	2	4	4	6	7	3	3	6	7	7	7	5	4	5	3	2	4	15.43	
Generations.....	17	16	13	14	13	15	16	17	15	15	17	16	16	15	15	14	16	15	13	17	18	15	15	15	15	16	15	15	15	463		
Excess in favor of 'fast'.....	4	1	4	4	5	6	2	4	6	4	3	4	1	2	1	5	2	1	5	7	3	6	4	2	4	8	2	2	3	107		
Third 10-day Period:																																
Fast:																																
Selections.....	1	5	5	8	4	4	5	4	4	3	5	6	5	7	5	4	5	6	4	6	5	6	5	6	4	5	6	7	6	2	22.70	
Generations.....	23	20	20	20	21	21	22	20	22	23	25	23	24	23	24	23	22	21	21	22	22	23	24	22	23	25	25	25	25	681		
Slow:																																
Selections.....	2	5	5	8	4	4	5	5	6	5	7	5	4	6	5	6	4	2	6	7	6	4	5	7	7	3	6	3	4	7	20.30	
Generations.....	21	18	17	15	18	20	18	19	17	19	22	23	20	20	21	20	19	19	23	23	20	21	23	23	22	24	24	609				
Excess in favor of 'fast'.....	2	2	3	5	2	1	6	3	3	3	0	3	4	3	1	1	1	3	3	2	0	4	2	2	2	3	2	1	72			
Fast:																																
Total Selections....	7	13	11	16	11	13	13	12	12	13	12	12	14	12	16	12	12	14	13	15	17	16	16	17	15	10	15	10	1814			
Total Generations..	63	57	55	57	64	64	65	61	58	63	62	58	61	60	61	57	56	58	60	72	63	65	62	62	65	68	63	66	59	1814		
Slow:																																
Total Selections....	9	14	8	18	10	13	11	10	16	17	21	14	13	11	16	12	12	16	15	13	17	20	15	9	15	10	13	15	15	15	15	1604
Total Generations..	56	51	48	45	49	53	50	55	50	51	54	58	53	51	53	53	50	53	48	58	55	53	57	56	56	58	60	58	60	1604		
Excess in favor of 'fast'.....	7	6	7	12	8	11	14	10	11	7	11	4	5	10	7	6	7	3	5	12	14	5	10	9	5	8	12	7	8	-1	240	
'fast'.....																																8.00

at random the animals for daily transfer to the fresh slides. But this method opened the possibility of the 'personal equation' becoming so important a factor that I rejected it. In place of it I devised what I have called the method of 'balanced selection.' Balanced selection is the process of compensating for the effect of every selection that one is compelled to make by making the reverse selection at the next opportunity; in other words, one makes the same number of plus and minus selections in any given line during each successive time interval adopted. Thus, if on a given day one makes a plus selection, at the next oppor-

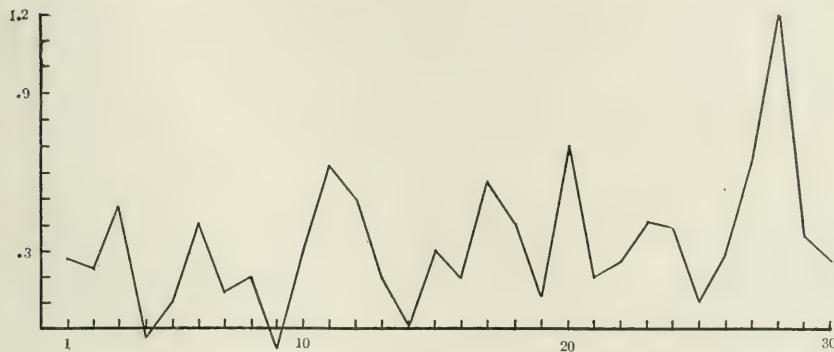


Fig. 2. Curve of the daily differences between the average number of generations per line produced by each of the two sets of lines while under selection in opposite directions (Exp. 1, part 1). The ordinates show the average differences (in fractions of a generation) in favor of the fast selected lines; the abscissæ are the days.

tunity he would make a reverse, or minus selection. In order to be able to tell at a glance whether to make a plus or minus selection the character of the selection to be made was also recorded on the slide.

In addition to carrying out balanced selection, in a portion of the lines reversed selection was practiced; that is, the fast lines were now subjected to minus selection, the slow lines to plus selection. Of course, if the observed difference in rate is hereditary and has resulted from selection, then there is no logical reason why the same result should not be obtained a second time, so that the difference would disappear, or eventually be

replaced by a reversed difference. The experiment with balanced and reversed selection is that described next.

*Experiment 1-A* Balanced selection and reversed selection, December 4 to December 23, 1913.

This experiment was undertaken to test by the methods set forth above whether the difference in fission rate shown at the end of thirty days of opposite selection by the two sets of lines in Experiment 1, part 1, were hereditary. In the first place fifty of the slow lines and fifty of the fast lines were subjected

TABLE 2

*Experiment 1-A: Actual number of generations per ten lines per ten-day period for the 50 fast and 50 slow lines of Experiment 1, Part 1, when subjected to balanced selection*

NUMBER OF LINES	11 TO 20	21 TO 30	31 TO 40	71 TO 80	91 TO 100	AVERAGE PER LINE PER 10-DAY PERIOD	AVERAGE PER LINE PER DAY
First 10-day Period:							
Fast lines.....	152	160	161	160	143	15.52	1.552
Slow lines.....	155	158	167	148	136	15.28	1.528
Differences in favor of the fast lines.....	-3	2	-6	12	7	0.24	0.024
Second 10-day Period:							
Fast lines.....	90	95	88	99	100	9.44	0.944
Slow lines.....	88	89	87	97	86	8.94	0.894
Differences in favor of the fast lines.....	2	6	1	2	14	0.50	0.050

to balanced selection for twenty days. Table 2 gives the actual number of generations that these fifty fast and slow lines produced. The sets are divided into groups of ten lines each.

The average difference of fission rate per line per day for the whole thirty days of Experiment 1, part 1, while selection was in progress was 0.267 generation in favor of the fast lines. For the twenty days of balanced selection it was 0.037 generation. This would seem to indicate that the direct selection of Experiment 1, part 1, had produced a very slight heritable difference in fission rate between the two sets, especially since the difference 0.05 generation of the second ten-day period of balanced

selection is considerably larger than for the first, 0.024 generation; and further it suggests the conclusion that if direct selection were practiced long enough a greater difference might be established.

TABLE 3

*Experiment 1-B: Actual number of generations per ten lines per ten-day period for the fifty fast lines and fifty slow lines subjected to reversed selection ('fast' lines selected now for slow fission, 'slow' lines for fast fission)*

NUMBER OF LINES	1 TO 10	41 TO 50	51 TO 60	61 TO 70	81 TO 90	AVERAGE PER LINE PER 10-DAY PERIOD	AVERAGE PER LINE PER DAY
First 10-day Period:							
Slow lines, <i>plus</i> selected:							
Selections.....	29	42	47	55	48		
Generations.....	157	187	174	166	180	17.28	1.73
Fast lines, <i>minus</i> selected:							
Selections.....	36	42	35	39	35		
Generations.....	147	160	150	137	145	14.78	1.48
Difference in favor of lines now plus selected.	10	27	24	29	35	2.50	0.25
Second 10-day Period:							
Slow lines, <i>plus</i> selected:							
Selections.....	24	28	23	38	25		
Generations..	96	108	88	110	96	9.96	1.00
Fast lines, <i>minus</i> selected:							
Selections.....	22	21	17	34	33		
Generations.....	95	93	92	93	95	9.36	0.94
Difference in favor of lines now plus selected.	1	15	-4	17	1	0.60	0.06

Reversed selection. Table 3 gives the actual number of fissions of the fifty fast and slow lines under reversed selection. Again the lines of each set are divided into groups of ten each.

Table 3 shows that reversed selection has made the average fission rate of the fifty slow lines faster than the average fission rate of the fifty fast lines. Now, as pointed out above, if the observed difference in fission rate is hereditary and has resulted from selection this result might logically be expected to follow from reversed selection. The net result of both phases of this

Experiment 1-A, while favorable to the production of an hereditary effect through selection, was to show that opposite selection must continue for a much longer period before the nature of the result can be established beyond controversy.

*Experiment 1, part 2.* Continued opposite selection, December 4 to December 23, 1913.

While Experiment 1-A was in progress certain fast and slow lines of Experiment 1, part 1, were kept under direct selection as a precaution against the possibility that selection in that experiment had not yet produced heritable differences in the fission rate. For this purpose fast lines 6, 30, 43, 45, 60, 73, 76, 78 and 100 and slow lines 3, 4, 9, 41, 53, 71 and 85 were chosen. During the first of these two ten-day periods the animals were transferred to fresh slides every twenty-four hours and during the second ten-day period, every forty-eight hours. The actual number of generations produced by each of these lines, during the two ten-day periods is shown in table 4.

TABLE 4

*Experiment 1, Part 2: Actual number of generations and selections per line per ten-day period, with the excess of generations in favor of the 'fast.' Direct selection of the fourth and fifth ten-day periods of Experiment 1.*

LINES NUMBER	FAST SLOW	6 3	30 4	43 9	45 41	60 53	73 71	76 85	78 100	AVERAGES
omitted in aver- ages										
<b>Fourth 10-day Period:</b>										
Fast:										
Selections.....	3	6	4	4	4	4	7	2	3	
Generations.....	14	16	18	18	17	20	17	18	18	17.14
Slow:										
Selections.....	2	5	3	6	5	3	5			
Generations.....	12	11	13	16	18	15	18			14.71
Excess in favor of 'fast'....	2	5	5	2	-1	5	-1			2.43
<b>Fifth 10-day Period:</b>										
Fast:										
Selections.....	1	3	1	3	3	3	2	3	2	
Generations.....	9	10	9	10	10	8	9	9	9	9.28
Slow:										
Selections.....	2	2	2	2	2	2	3			
Generations.....	7	4	7	5	4	5	8			5.71
Excess in favor of 'fast'....	2	6	2	2	6	3	1			3.57

This experiment is simply a connecting link between part one and part three of Experiment 1. There is a slight increase in the difference between the average number of generations produced by the fast and slow lines during the first and second ten-day periods. The fourth and fifth ten-day periods of figure

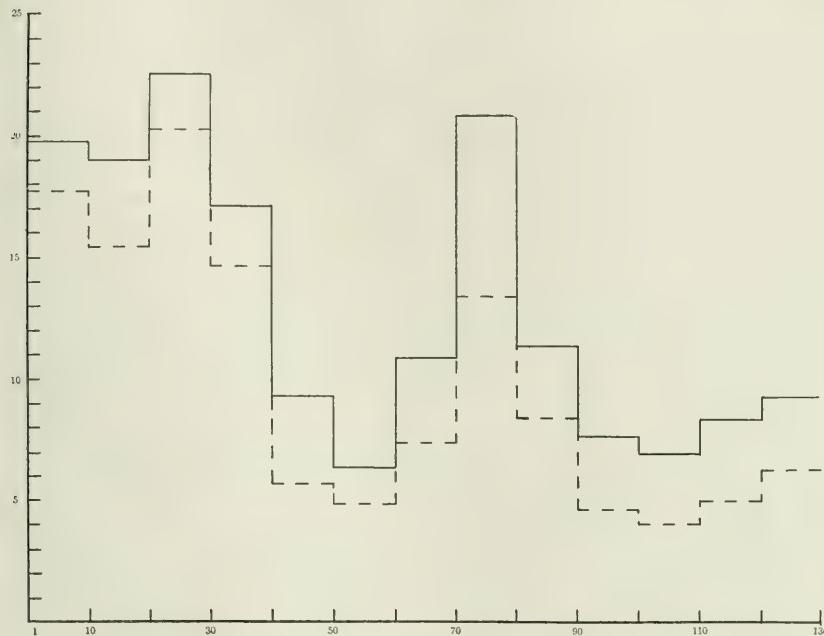


Fig. 3 Polygon of the average number of generations produced, per line, by the fast and slow selected sets of lines of Experiment 1, while the two were undergoing selection in opposite directions; averaged for ten-day periods. The polygon shows this average for each of all the 13 consecutive ten-day periods of Experiment 1. The continuous line shows the average for the fast lines, the broken one the average for the slow lines. The ordinates give the average number of generations produced and the abscissae the number of days since the beginning of the experiment—the successive ten-day periods.

3 show the average number of generations per line per ten-day period for this part of the first experiment and its first three ten-day periods show it for the first part; while figure 4 is the curve of the daily differences between the average number of generations produced by each fast and each slow line during these two ten-day periods.

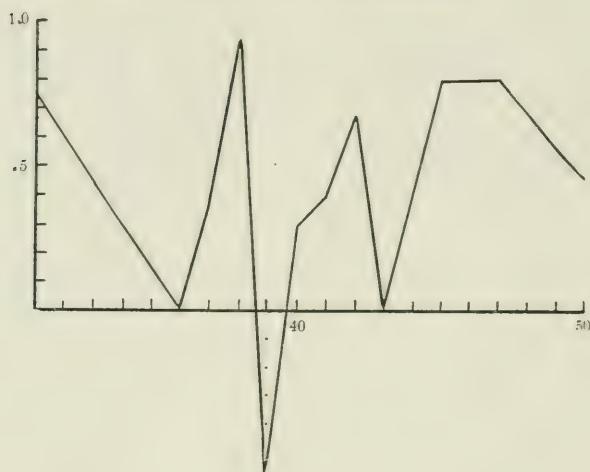


Fig. 4 Curve of the daily differences between the average number of generations per line produced by the fast selected set and the slow selected set during the fourth and fifth ten-day periods of opposite selection in Experiment 1, (i.e., Exp. 1, part 2.) The ordinates give the daily differences, the abscissae give the days.

*Experiment 1, part 3.* Continued opposite selection, December 24, 1913, to January 22, 1914.

It will be noticed from table 1 and figure 3 that the differences between the average number of generations produced by the fast lines and the slow lines during the three consecutive ten-day periods of Experiment 1, part 1, are 2.03, 3.57 and 2.40. That is, each fast line produced, on the average, 0.267 generation more per day than each slow line during these thirty days. Table 4 and figure 3 show that the corresponding differences for the fourth and fifth ten-day periods of Experiment 1, were 2.43 and 3.57 which gives a daily average difference of 0.300 generation per line. This difference, in favor of the average fast line is slightly (0.033 generation), greater than the corresponding difference of Experiment 1, part 1 (0.267 generation). Does this mean that on the average, selection is gradually increasing this difference? Experiment 1-A indicates that opposite selection had produced a heritable difference in the average fission rate of the two sets of lines; may this heritable difference be increased by further opposite selection?

To answer this question the first seven of the fast lines of Experiment 1, part 2, were each increased (by division) to four and from the eighth two lines were derived, giving thirty fast lines in all. Each of the slow lines of Experiment 1, part 2, was likewise increased to four, with the exception of the last one which was increased to six, thus giving thirty slow lines. These two sets of lines were then selected for fast and slow rates of fission. During the three ten-day periods of this part of Experiment 1, the selection and transfer of animals to fresh slides was made every forty-eight hours instead of at the close of every twenty-four hour interval as heretofore.

At the end of the first ten-day period the eight fast lines which had produced the highest number of generations were selected and thirty lines were derived from them, care being taken that each group of ten lines, the 'fast' occupants of a single moist chamber, were represented in the new set. Also eight of the slow lines which had produced the smallest number of generations were selected and from them thirty lines for continued slow selection were derived. The same precaution was taken in reference to the distribution of the selected lines among the three moist chambers. These two sets of lines were then selected for fast and slow fission rates during the second ten-day period. Finally this same method of reduplication of the fastest and the slowest lines and the subsequent continued selection of the individual variations of fission rate was followed through the third ten-day period.

This method of double selection was devised with the purpose of crowding as much selection into the experiment as possible, in the attempt to determine whether continued selection would gradually increase the previously established difference between the average rates of fission of the two sets of lines. It will be remembered that the difference of average fission rates between the two sets of lines at the end of Experiment 1, part 2, was 3.57 generations per line per ten-day period as shown by the fifth ten-day period of figure 3 and by table 4. The sixth, seventh and eighth ten-day periods of figure 3 show those differences for the three ten-day periods of the present part of Experi-

ment 1. For the first period of this part (sixth period of the entire experiment) the difference was 1.53 generations per line per ten-day period, for the next it was 3.41 generations and for the last it was 7.51 generations. The small difference of 1.53 between fast and slow in period 6, following upon a period when the difference was 3.57, is due to a slowing of the fission rate in all lines during period 6, owing probably to low temperature, and perhaps partly also to one of the rhythms emphasized by Woodruff and his colleagues. The percentage of difference in proportion to the total average fission rate is in reality greater in period 6 than in preceding periods. Thus, for period 4 the average number of fissions for all sets was 15.925, and the difference between the fast and slow was 2.43—this difference being thus 15.25 per cent of the average rate. In period 6, the small difference 1.53 is actually 27.34 per cent of the average rate for all. Table 5 gives the actual number of generations produced by each of the lines during the three consecutive ten-day periods, the number of selections that were made in each line and the average difference per line for each ten-day period.

Table 5 and figure 3 demonstrate in this experiment a continued increase of the difference between the average rates of fission of the two sets of lines. As has each of the previous experiments, so also does this one show that the fission rate within the clone may be changed by selection. During its three ten-day periods each fast line produced on the average 0.415 generations more per day than each slow one. On only one day during the thirty days of this experiment did the slow lines produce more generations than the fast lines and on that day only an average of 0.3 generation per line, as shown by figure 5. The two sets indeed hardly overlap at all in their rates, practically all of the fast set being faster than any of the slow set. This is shown by the curves of variation of the two sets in figure 6. Only one slow line produced as many generations as the slowest fast line.

TABLE 5

*Experiment 1, Part 3: Actual number of generations and of selections per ten-day period per line of the third part of Experiment 1, that is, the sixth, seventh and eighth ten-day periods of continuous opposite selection, with the excess in generations produced in favor of the fast-selected set*

LINES NUMBER	1 TO 10	11 TO 20	21 TO 30	TOTAL	AVERAGE PER LINE PER TEN-DAYS
Sixth 10-day Period:					
Fast:					
Selections.....	13	14	14		
Generations.....	62	65	64	191	6.36
Slow:					
Selections.....	5	8	11		
Generations.....	44	48	53	145	4.83
Excess in generations in favor of the fast.....	18	17	11	46	1.53
Seventh 10-day Period:					
Fast:					
Selections.....	28	22	21		
Generations.....	121	101	104	326	10.87
Slow:					
Selections.....	23	25	23		
Generations.....	69	88	67	224	7.46
Excess in generations in favor of the fast.....	52	13	37	102	3.41
Eighth 10-day Period:					
Fast:					
Selections.....	32	42	41		
Generations.....	168	222	236	626	20.87
Slow:					
Selections.....	31	31	36		
Generations.....	132	133	134	399	13.30
Excess in generations in favor of the fast.....	36	89	102	227	7.56
Fast:					
Total for 30 days.....	351	388	404	1143	38.10
Slow:					
Total for 30 days.....	247	271	254	772	25.73
Difference.....	104	117	150	371	12.37

*Experiment 1-B.* Balanced selection, January 23 to April 22, 1914.

At the close of Experiment 1, part 3, the two sets of lines of that experiment had been under continuous opposite selection

for eighty days, and the average difference per line per day had increased from 0.267 generation for the first thirty days to 0.415 generation for the last thirty days of that period. To test the permanence of this apparent effect of selection, to determine the answer of our experiments to the question "Can we get from a single genotype by selection two genotypes that differ charac-

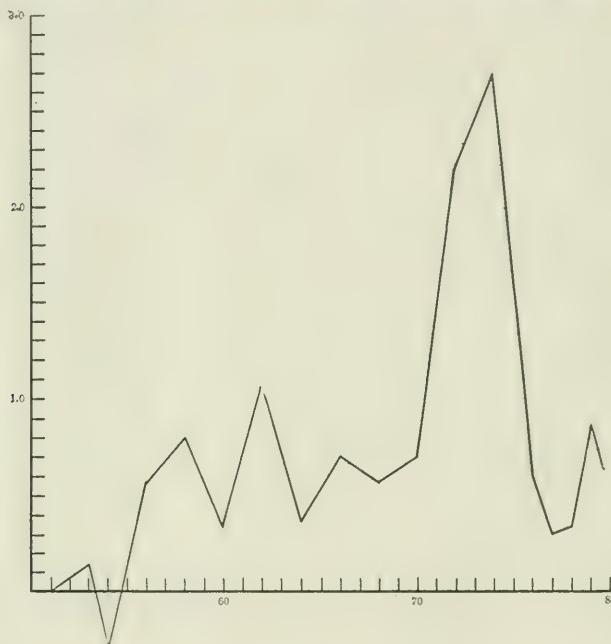


Fig. 5 Curve of the daily difference between the average number of generations per line produced by the fast set and the slow set during the sixth, seventh and eighth ten-day periods of opposite selection in Experiment 1 (Exp. 1, part 3). The ordinates give the daily excess in favor of the fast-selected lines, the abscissae give the days.

teristically from each other under identical conditions; and that retain these differences from generation to generation?"—the lines were now subjected to the test of balanced selection. For this purpose all the thirty fast and thirty slow lines which were in progress at the close of Experiment 1, part 3, were continued. In order to make the test thorough it was prolonged through nine consecutive ten-day periods, or ten-days longer than the

lines had been subjected to opposite selection. During this whole experiment the animals were transferred to fresh slides daily as described at the beginning of this paper.

The results of Experiment 1-B, are so important that they are set forth in some detail in table 6. This table gives the actual number of generations produced by each fast and each

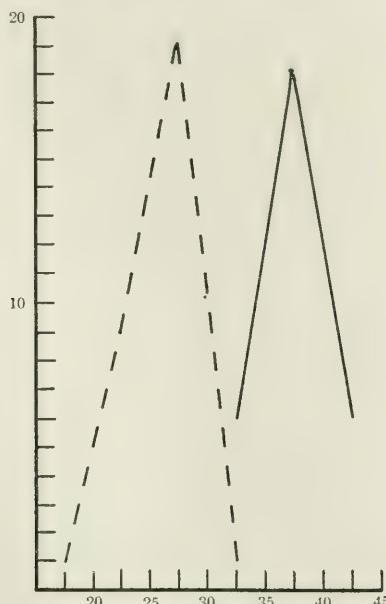


Fig. 6 Curves of variation in actual number of fissions of the two sets of lines of Experiment 1, Part 3. The ordinates give the number of lines, the abscissae the number of generations produced during the thirty days. The continuous line is the curve of the fast-selected set, the broken one is that of the slow-selected set.

slow line during each ten-day period of the experiment, and the difference between them. This difference is given as a positive quantity when the fast line has produced more generations than the corresponding slow one and negative when the reverse is the case. The latter occurred only thirty-one times among the whole two hundred and seventy differences. In twelve of the lines it did not occur at all; in nine lines, once; in six lines, twice;

TABLE 6  
Experiment I-B: Actual number of generations per ten-day period for the nine consecutive ten-day periods of balanced selection which immediately followed the eighty days of direct selection of these parts of Experiment I. The differences in number of fissions in favor of the fast lines are given also.

LINES NUMBER	First 10-day Period:										Second 10-day Period:										Third 10-day Period:										Fourth 10-day Period:										Fifth 10-day Period:										Sixth 10-day Period:									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE																												
Fast, Generations	17	21	15	18	15	16	19	17	13	12	14	17	13	9	9	12	14	11	11	11	12	9	12	13	10	13	12	14	11	12	402	13.40																												
Slow, Generations	15	12	16	15	14	11	14	11	11	13	12	8	7	9	10	12	6	9	8	8	11	9	8	6	8	9	9	9	9	308	10.26																													
Difference.....	2	9	-1	3	1	5	6	2	-1	2	0	6	0	-1	0	8	2	3	3	1	0	4	4	4	5	3	5	2	3	94	13.27																													
Second 10-day Period:																																																												
Fast, Generations	8	7	8	6	8	6	6	7	8	6	7	3	8	4	7	9	6	7	6	5	5	3	5	5	4	6	2	3	3	5	5	1.14																												
Slow, Generations	8	8	5	6	5	4	6	5	5	5	7	5	5	6	5	5	3	5	5	3	5	5	4	6	2	3	2	3	0	50	1.67																													
Difference.....	0	-1	3	0	3	2	0	2	3	1	0	-2	3	-1	1	4	1	4	1	0	2	2	4	2	3	2	3	0	3	50	14.55																													
Third 10-day Period:																																																												
Fast, Generations	10	10	8	7	8	10	12	8	6	8	6	7	6	8	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	237	7.99																												
Slow, Generations	8	9	8	7	6	10	8	6	8	6	8	5	7	6	8	4	7	8	6	4	5	6	5	6	3	5	4	4	4	190	6.33																													
Difference.....	2	1	0	0	2	-2	2	4	2	-2	1	0	0	1	0	0	2	2	-1	-2	3	4	3	2	4	4	4	3	47	1.57																														
Fourth 10-day Period:																																																												
Fast, Generations	9	12	10	9	13	11	9	10	11	12	9	9	10	6	8	6	7	8	7	8	6	9	7	9	10	10	9	9	11	272	9.06																													
Slow, Generations	10	10	7	10	8	11	7	6	11	8	8	4	5	7	6	10	5	7	9	7	5	4	5	6	8	5	7	5	6	9	216	7.02																												
Difference.....	-1	2	3	-1	5	0	2	4	0	4	1	5	5	-1	2	-4	2	1	-2	1	3	2	4	1	1	5	3	4	3	56	1.86																													
Fifth 10-day Period:																																																												
Fast, Generations	11	12	15	9	13	15	13	15	15	12	10	12	7	12	9	5	9	9	10	11	8	11	13	7	9	10	9	9	14	329	10.96																													
Slow, Generations	12	12	4	8	10	14	9	12	12	7	8	6	4	8	7	8	7	7	7	6	5	5	5	5	5	5	6	8	11	240	8.00																													
Difference.....	-1	0	11	1	3	1	6	9	3	3	5	2	6	3	4	2	-3	2	2	3	5	3	6	8	-1	2	-2	1	2	3	89	2.96																												
Sixth 10-day Period:																																																												
Fast, Generations	14	11	15	11	10	14	16	16	15	12	9	9	9	14	11	8	12	10	8	13	13	9	8	10	10	14	349	11.63																																
Slow, Generations	14	12	12	10	12	11	13	9	11	11	10	6	6	9	13	11	8	6	8	3	6	0	9	5	11	6	4	12	263	8.77																														
Difference.....	0	-1	3	1	-2	3	3	7	5	4	2	3	3	0	1	0	0	5	2	4	7	2	13	4	4	4	4	4	6	2	86	2.86																												

Seventh 10-day																						
Period:																						
Fast, Generations	11	11	13	16	12	14	13	11	11	9	9	13	10	12	13	11	10	9	14	7	7	12
Slow, Generations	9	10	10	12	13	14	12	5	8	12	8	10	9	10	10	8	7	7	5	4	6	10
Difference.....	2	1	3	4	-1	0	1	6	3	-1	1	-1	4	0	2	5	4	3	2	9	3	11
Eighth 10-day																						
Period:																						
Fast, Generations	11	12	13	13	14	15	15	14	13	14	11	13	13	13	14	11	11	12	11	12	11	14
Slow, Generations	8	11	10	9	14	16	15	5	11	13	11	14	13	10	13	11	10	5	3	4	6	8
Difference.....	3	1	3	4	0	-1	0	9	2	1	0	-1	0	1	0	1	2	6	9	4	6	3
Ninth 10-day																						
Period:																						
Fast, Generations	13	13	14	17	18	17	14	14	8	10	13	13	12	8	10	12	11	9	9	13	10	10
Slow, Generations	7	8	10	14	11	13	12	12	12	14	11	12	14	12	6	9	6	5	7	8	4	11
Difference.....	6	5	4	3	7	4	2	2	-4	-4	2	-2	-4	2	3	5	4	2	5	4	3	11
Differences for the whole 90-days.....	13	17	23	15	18	12	21	49	16	5	4	16	26	0	14	10	20	25	15	32	25	39

In this table the numbers in the 'difference' columns when positive indicate that the fast line in question has produced so many more generations than the corresponding slow one; thus during the first ten-day period fast line No. 1, produced 2 more generations than slow line No. 1. When the number in the difference column is negative the slow line has produced more generations than the corresponding fast one during the ten-day period; this is the case for slow line No. 10 during the first ten-day period.

The average difference in favor of the fast, per ten-day period during this experiment, is 2.51 generation per line.

in two lines, three times; and in one line four times. When the differences between the number of generations produced by the corresponding lines during the whole ninety days were ascertained it was found that in *not a single case had the slow line produced more generations than its fast one*. And in only two lines was the excess of the fast over the slow probably too small to be significant (fast line ten produced only five more generations than slow line ten, and the two lines number fourteen produced the same number of generations). Furthermore during each ten-day period the *total number of generations produced by all the thirty fast lines was much larger than that produced by the slow lines, and the average number of generations per line per ten-day period was uniformly greater for the fast than for the slow lines*. Also the per cent of the difference in proportion to the total number of generations produced by both sets was calculated and found to be remarkably uniform; these percentages are shown in the extreme right hand column of table 6. Finally, the average number of generations per line per ten-day period for each set of lines is plotted as a polygon in figure 7-a, giving a graphic representation of that phase of table 6.

Figure 8 gives the curve of the daily differences between the average number of generations produced by each fast and each slow line. On only three days during the whole ninety days of this balanced selection experiment was this difference too small to be significant (on the eighth and sixteenth of February, 1914, both sets of lines produced the same number of generations and on February 18, 1914, the difference was 0.03 in favor of the slow set). The average per day for each fast line, including the three instances just cited, was 0.251 generation greater than each slow line. From table 6 and from figures 7 and 8 it is therefore evident that, when measured by the test of balanced selection, the eighty days of opposite selection had produced a difference of fission rate between the two sets of lines that is heritable. Furthermore, analysis of the daily records shows that this result is not due to the chance isolation of a 'mutating' line in either set of lines and the subsequent development of the thirty lines of

the set from that one, for we have four lines of the fast set that run all the way through and three of the slow lines that do the same. Table 7 shows the total number of generations produced by each line of both sets. For the fast set these totals vary from

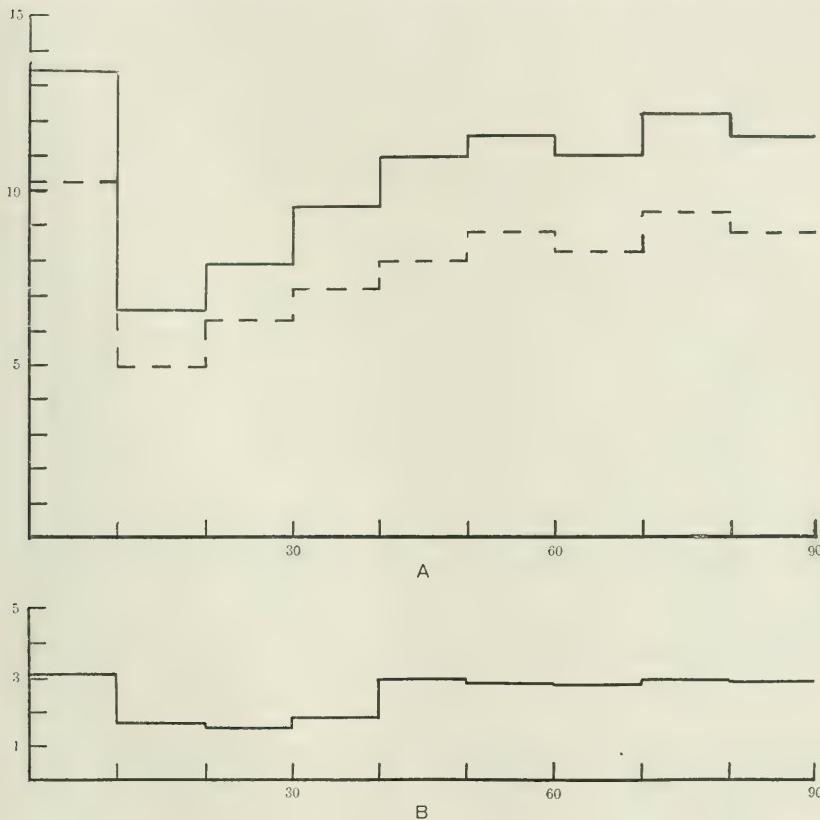


Fig. 7-a Polygons of the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-B during its nine consecutive ten-day periods of balanced selection. The continuous line shows the averages for the fast set, the broken line the averages for the slow set. The ordinates show the differences of the averages, the abscissae the ten-day periods.

Fig. 7-b Curve of the difference between the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-B during its nine consecutive ten-day periods of balanced selection. The ordinates show the differences of the averages in favor of the fast set, the abscissae the consecutive ten-day periods.

TABLE 7

*Experiment 1-B: The total number of generations produced by each fast and slow line during balanced selection for the ninety days of the experiment with the difference between the number of generations produced by each fast line and its corresponding slow line. Also the sum of the number of generations produced by each fast line and its corresponding slow line, and the percent that the differences are of the sums*

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30											
Fast, number of generations in 90 days.....	104	109	111	106	111	116	117	114	103	101	95	91	96	73	92	91	79	83	76	91	82	73	88	96	84	95	86	89	83	98											
Slow, number of generations in 90 days.....	91	92	92	91	93	104	96	65	87	96	81	75	70	73	78	81	59	68	61	59	50	48	49	60	59	54	71	53	50	69											
Totals.....	195	201	193	197	204	220	213	173	190	197	176	166	166	146	170	172	138	141	137	150	132	121	137	156	143	149	157	142	133	164											
Excess in favor of fast.....	13	17	29	15	18	12	21	49	16	5	14	16	26	0	14	10	20	25	15	32	32	25	39	36	25	41	15	36	33	29											
Per cent that excess is of total....	47	45	15	52	7	61	8	82	5	45	9	85	27	37	8	42	2	53	7	55	9	63	15	66	8	23	5	81	14	38	17	48	57	51	9	55	35	24	81	17	68

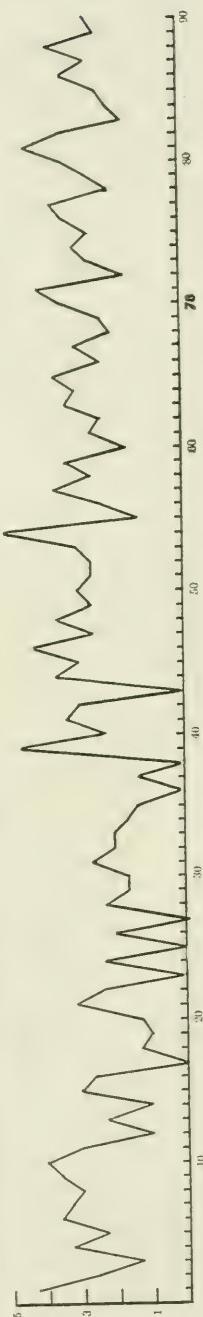


Fig. 8 Curve of the daily difference between the average number of generations per line produced by the fast and slow sets of lines of Experiment 1-B during its ninety days of balanced selection. The ordinates show the differences of the daily averages in favor of the fast set; the abscissae are the ninety days. The ordinates of this curve, averaged for ten-day periods, give figure 7-b.

seventy-three to one hundred and seventeen and for the slow set from forty-eight to ninety-six with the exception of one line which produced one hundred and four generations. The first ten lines of both sets each produced a much larger number of generations than the remainder of the lines of its own set. These lines were the occupants of a single moist chamber, so that there was probably some environmental difference distinguishing this moist chamber from the other two. That environmental differences have a marked effect on the fission rate of infusoria has of course been shown by many writers.

Figure 9-a gives the curves of variation in number of generations produced by each fast and each slow line during balanced selection. The curve for the slow lines shows an apparent bimodality. To determine whether this might be attributable to the environmental difference suggested by table 7 these same variation curves were plotted for the first ten lines of each set alone. These curves are shown as figure 9-b; the curves are mutually exclusive. Table 7 shows that only one slow line among these ten produced more generations than the slowest one of these first ten fast lines. Figure 9-c gives the curves of variation for fast and slow lines 11 to 30, the occupants of the other two moist chambers. Only four of these twenty slow lines produced more generations during the ninety days of balanced selection than the slowest fast line.

It must be borne in mind in this connection that these "two sets of thirty lines" are parts of the same clone. That is, we have got here, from a single genotype by selection two genotypes that differ characteristically from each other under identical conditions; and that retain these differences from generation to generation.

*Experiment 1, part 4.* Further opposite selection, January 23 to March 14, 1914.

While Experiment 1-B was in progress the original lines were continued under direct selection for two reasons: 1) As a precaution against the possible failure of the difference of fission

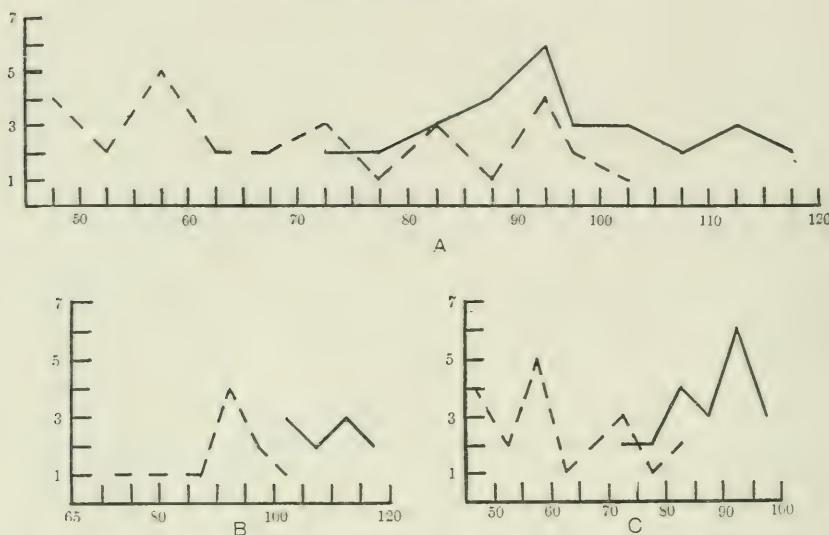


Fig. 9-a. Curves of variation of the lines of Experiment 1-B, the ninety-days of balanced selection. The ordinates give the number of lines, the abscissae the number of generations produced. The continuous line is the curve of the fast set, the broken line that of the slow set.

Fig. 9-b Curves of variation of the first ten fast and first ten slow lines of Experiment 1-B (balanced selection). The ordinates represent numbers of lines, the abscissae, generations. The continuous line is the curve of the fast set, the broken one the curve of the slow set.

Fig. 9-c Curves of variation of the last twenty fast and last twenty slow lines of Experiment 1-B (balanced selection). The ordinates represent numbers of lines, the abscissae, generations. The continuous line is the curve of the fast set, the broken one the curve of the slow set.

rate to survive long continued balanced selection (Experiment 1-B); 2) To discover what would be the effect if selection continued further on these lines. During the first three ten-day periods there was no further reduplication of extreme lines and the slides were changed daily. At the end of the third ten-day period the fastest lines of the fast set and the slowest lines of the slow set were again chosen for reduplication and continued selection, as described for Experiment 1, part 3. This was the only time this was done during the present experiment. The slides were changed every forty-eight hours during the fourth and fifth ten-day periods. The ninth to the thirteenth ten-day

periods of figure 3 (which gives the average fission rates of the two sets of lines of the entire first experiment for the one hundred and thirty days they were selected), give the average fission rates of the lines of this part of Experiment 1. Table 8 gives the actual number of generations per 30 lines as well as the average number of selections and generations per line that occurred and the differences in the average fission rates.

It has been pointed out that, on the average, for the first three ten-day periods of Experiment 1 each fast line produced

TABLE 8

*Experiment 1, Part 4: Actual and average number of generations and of selections per 30 lines per ten-day period during the ninth to the thirteenth ten-day periods of continuous opposite selection of the lines of Experiment 1*

TEN-DAY PERIODS	NINTH	TENTH	ELEVENTH	TWELFTH	THIRTEENTH	TOTAL	AVERAGE PER LINE
<b>Fast lines:</b>							
Average number of selections per line.	2.57	2.47	32.3	1.63	2.30		
Total number of generations	341	230	206	250	279	1306	43.53
Average number of generations per line	11.37	7.67	6.87	8.33	9.30		
<b>Slow lines:</b>							
Average number of selections per line.	1.57	2.33	1.36	1.33	1.97		
Total number of generations	251	139	121	149	189	849	28.30
Average number of generations per line	8.37	4.63	4.03	4.96	6.30		
Actual excess of generations in favor of fast lines.....	90	91	85	101	90	457	15.23
Average excess per line in favor of fast lines.....	3.00	3.04	2.84	3.37	3.00		3.05

0.267 generation more per day than each slow line. During the fourth and fifth ten-day periods each fast line produced, on the average 0.300 generation more per day than each slow line. During the sixth, seventh and eighth ten-day periods this daily average difference per line was 0.415 generation. Table 8 shows that during the ninth, tenth, eleventh, twelfth and thirteenth ten-day periods it was 0.305 generation, which is considerably smaller than the 0.415 generation difference of the sixth, seventh and eighth ten-day periods of Experiment 1. But, as in a previous case, this is due merely to the fact that the average fission rate for all lines has decreased; relative to this average fission rate the difference between fast and slow lines has not decreased, but on the contrary has increased. For part 1 of Experiment 1, the difference between the fast and the slow lines in number of generations produced was 6.9 per cent of the total number of generations produced by all; for part 2 it was 12.8 per cent; for part 3, 19.3 per cent, and for part 4 it was 21.2 per cent. Consequently, throughout the entire period of selection (thirteen ten-day periods), the proportional difference between fast and slow lines has steadily increased.

Figure 10 further emphasizes the genuineness of the difference of fission rate between these two sets of lines for Experiment 1, part 4. It shows the curves of variation of these two sets of lines of that experiment.

*Experiment 1-C. Mass culture and balanced selection, March 17 to May 4, 1914.*

In order to demonstrate as conclusively as possible whether the apparent average difference of fission rate between the two sets of lines was hereditary or not, it was decided to subject them to a period of mass culture and then to balanced selection. This was to determine whether the average difference of fission rate had survived the mass culture treatment. On March 4, 1914, all the animals remaining in the 'fast' concavities after the transfer of the chosen individuals had been made to fresh slides were placed, unwashed in a single circular glass dish  $3\frac{3}{4}$  inches in diameter and 2 inches deep in 50 cc. of  $\frac{1}{32}$  per cent

Horlick's malted milk; and all those remaining in the 'slow' concavities were similarly treated. These two mass cultures were placed side by side on the laboratory table and allowed to propagate for twelve days. Every three days 25 cc. of boiled and cooled spring water was added to each to compensate for evaporation. The animals placed in these mass cultures were taken from Experiment 1-B, and hence had been subjected to opposite selection for eighty days and balanced selection for

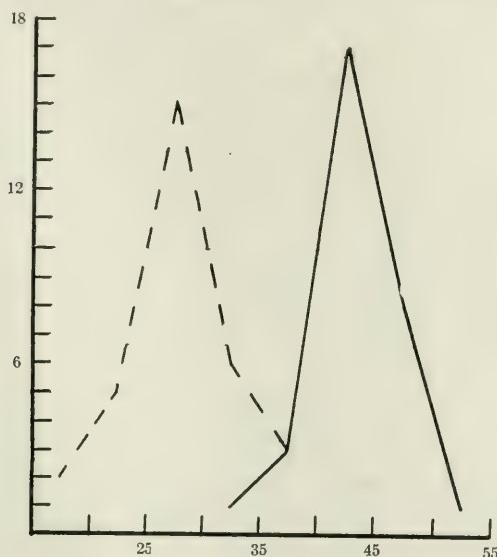


Fig. 10 Curves of variation of the lines of Experiment 1, Part 4 (continuous selection). The ordinates give numbers of lines, the abscissae the generations produced during the fifty days of the experiment. The continuous line is the curve of variation of the fast set, the broken one the curve of the slow set.

forty days; they were now allowed to remain in mass cultures for twelve days.

On March 17, 1914, thirty individuals were taken from the mass culture of fast lines and isolated on slides and thirty individuals were also isolated from the mass culture of slow lines. These sixty lines were then subjected to balanced selection for a period of fifty days, the transfer to fresh slides being made daily.

In order to be sure of the uniformity of the bacterial content of the slides, on March 18, an equal small quantity of the liquid from each of the mass cultures was added to the fresh medium. It was added two or three drops at a time and each few drops carefully studied in a watch glass under the binocular before it was added to the fresh culture medium. On March 20, each animal transferred to the fresh slides was washed in a watch glass in a mixture of equal quantities of the culture medium from the mass cultures instead of being washed in fresh culture medium.

Now at the end of this fifty-day period of balanced selection the two sets had experienced *eighty* days of *opposite selection* immediately followed by *one hundred and two days* of *no selection*. If the difference remains after this test it is evident that *selection has in this case produced an heritable difference in fission rate within the clone*. Table 9 shows that this difference of average fission rate does persist. Figure 11 shows the rate of division of the two sets of lines, averaged for ten-day periods, during this balanced selection test.

*Experiment 1-D.* Reversed selection, April 13 to June 1, 1914.

A second experiment in reversed selection was started on April 13, 1914, from lines derived from Experiment 1-B, the ninety-day balanced-selection experiment described above. Hence they had been subjected to eighty days of opposite selection and this was followed by eighty days of balanced selection before reversed selection was started. This experiment was prolonged through five ten-day periods with daily transfer to fresh slides and during the whole time the average fission rate of the fast-selected 'slow' lines was higher than that of the slow-selected 'fast' lines. And on the whole there was a gradual increase of this average difference as reversed selection proceeded. Here again selection has altered the fission rate and the new rates were hereditary. Figure 12 and table 10 show graphically the results of this experiment.

Attention is called in table 10 to the large average number of selections per line for the fifty days of this reversed selection.

TABLE 9  
*Experiment 1-C: Actual number of generations per ten-day period per line. Balanced selection after mass culture*

LINES NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	AVERAGE
First 10-day Period:																																
Fast, Generations...	12	12	12	14	14	15	15	12	13	13	13	13	13	13	13	13	12	12	13	11	14	11	10	10	11	11	11	12	11	363-6	11.9	
Slow, Generations...	8	10	13	9	11	9	13	14	10	10	9	10	12	14	7	13	9	12	10	8	12	11	10	11	9	12	11	10	11	318+6	10.8	
Excess in favor of fast lines.....																																
Second 10-day Period:	4	2	-1	3	3	5	2	-2	2	3	3	4	0	1	-1	6	-1	3	1	1	6	-1	-1	0	0	2	-1	0	2	0	1.1	
Fast, Generations...	10	13	10	12	12	12	11	11	9	10	11	11	10	10	11	9	10	10	10	9	8	4	9	11	12	9	11	10	10	307-4	10.10	
Slow, Generations...	8	10	9	8	9	9	8	12	3	3	7	7	7	10	11	7	9	7	6	9	6	7	8	9	10	3	10	12	7	7	238-6	7.73
Excess in favor of fast lines.....																																
Third 10-day Period:	4	3	1	4	3	3	4	-1	8	6	3	4	4	0	-1	4	0	3	4	1	3	1	-4	0	1	9	-1	-1	3	3	2.37	
Fast, Generations...	7	12	13	12	11	14	14	11	9	12	11	12	10	12	11	12	9	10	12	11	7	11	11	12	11	11	11	9	10	330+3	11.1	
Slow, Generations...	16	12	12	10	10	11	8	9	4	7	10	6	9	11	11	8	12	11	12	11	8	10	1	12	11	7	6	285-3	9.4			
Excess in favor of fast lines.....																																
Fourth 10-day Period:	-9	0	1	2	1	3	6	2	5	2	5	2	1	-1	4	-1	3	-4	-1	0	0	-1	3	1	11	-1	0	4	3	1.7		
Fast, Generations...	11	13	11	9	9	14	13	11	11	8	12	11	13	14	10	15	13	9	8	10	9	12	8	13	13	10	8	10	9	320+7	11.20	
Slow, Generations...	14	13	14	11	10	11	7	12	4	5	11	11	9	9	12	12	5	7	12	12	3	11	11	2	12	9	6	9	235-5	9.33		
Excess in favor of fast lines.....																																
Fifth 10-day Period:	-3	0	-3	-2	-1	3	6	-1	7	3	1	0	4	5	1	3	1	4	1	-2	-3	0	5	2	2	11	-2	-1	4	0	1.87	
Fast, Generations...	16	10	15	16	13	15	17	11	9	15	14	14	13	14	12	11	12	13	12	9	11	12	12	14	15	14	13	14	11	10	337+5	13.06
Slow, Generations...	14	8	13	13	12	14	7	11	10	11	11	12	14	12	10	11	15	12	6	15	12	13	13	12	13	13	10	9	14	355-3	11.73	
Excess in favor of fast lines.....																																

The column labeled "Surplus Selections" indicates that for instance during the first 10-day period, six more fast selections were made than slow selections in the fast lines. Therefore six is subtracted from the total number (363) of generations produced and the average 11.9 is gotten by dividing this remainder (357) by 30, the number of lines.

1.33

It is 13.74 selections per line for the fast-selected 'slow' lines and 12.79 selections for the slow-selected 'fast' lines. Now the average difference of fission rate per line per day for the eighty days of balanced selection immediately preceding the present experiment was 0.25 generation per line per day and as a result of the fifty days of reversed selection this difference was wiped out and

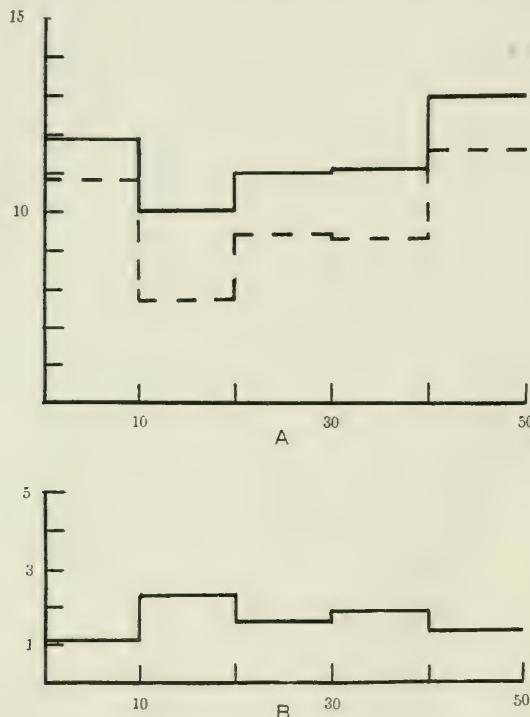


Fig. 11-a Polygon of the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-C (balanced selection for 50 days after 80 days of selection, 40 days of balanced selection and 12 days of mass culture). The continuous line shows the averages for the 'fast' set, the broken one the averages for the 'slow' set. The ordinates are the averages per ten-day periods, the abscissae, the ten-day periods.

Fig. 11-b Curve of the difference in favor of the fast lines between the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-C during its five consecutive ten-day periods of balanced selection after mass culture. The ordinates are the differences of the averages, the abscissae the consecutive ten-day periods.

an average difference of 0.27 generation established in the reverse direction.

If this difference shows itself to be heritable, it will appear that it is probably due to the large number of selections practised within the relatively short period of fifty days; it will indi-

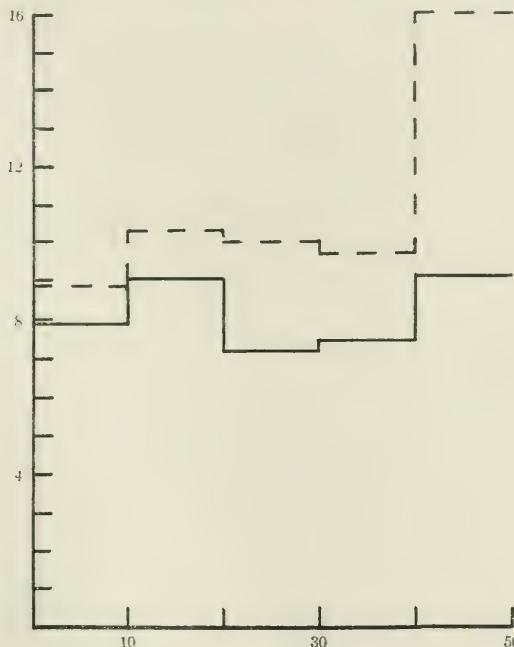


Fig. 12 Polygon of the average number of generations per line per ten-day period produced by the 'fast' and 'slow' sets of lines of Experiment 1-D (reversed selection). The continuous line of the curve of the former fast lines now slow-selected. The broken line is the curve of the former slow lines now fast-selected. The ordinates are the averages per ten-day periods, the abscissae the ten-day periods.

cate that the alteration of fission rate is proportional to the number of selections made, rather than the length of time over which the selections are distributed. This matter is tested in the next experiment.

*Experiment 1-E.* Balanced selection after reversed selection, June 2 to June 21, 1914.

TABLE 10

*Experiment 1-D: Actual number of generations and selections per 30 lines per ten-day period during the five ten-day periods of the second reversed selection of the lines of Experiment 1*

TEN-DAY PERIODS	FIRST	SECOND	THIRD	FOURTH	FIFTH	TOTAL	AVERAGE PER LINE
Fast Selected Slow Lines:							
Average number of selections.....	2.40	2.77	2.63	2.27	3.67		
Total number of generations	263	309	300	292	473	1637	54.57
Average number of generations.....	8.77	10.30	10.00	9.73	15.77		10.91
Slow Selected Fast Lines:							
Average number of selections.....	2.83	2.70	2.13	2.60	2.53		
Total number of generations	237	273	217	225	276	1228	40.93
Average number of generations.....	7.90	9.10	7.23	7.50	9.20		818
Actual excess in favor of the fast selected 'slow' lines....	26	36	83	67	197	409	13.64
Average excess in favor of the fast selected 'slow' lines....	0.86	1.20	2.77	2.23	6.56		2.73

The lines from the experiment in reversed selection (Experiment 1-D) just described were next continued under the balanced selection method for two ten-day periods in order to determine whether the differences in the reverse direction obtained in that experiment were true heritable differences of fission rate. The animals were transferred to fresh slides daily. Figure 13 shows the polygon of the average fission rates of these two sets of lines and table 11 gives the actual number of generations produced by each set. From these it appears that the average daily difference of fission rate per line between the two sets of lines is 0.38 generation in favor of the fast selected slow set.

It is therefore clear that reversed selection had really produced an heritable difference in fission rate in favor of the former slow lines; the relative rates of fission of the two sets was now reversed. As before remarked, this result is to be expected; the earlier experiments show that direct selection produces hereditary differences in one direction; reversed selection

in the same way produces hereditary differences in the opposite direction.

*General results of the first group of experiments.* The experiments thus far described have all dealt with parts of a single clone of *Styloonychia pustulata*; selection has been practised on

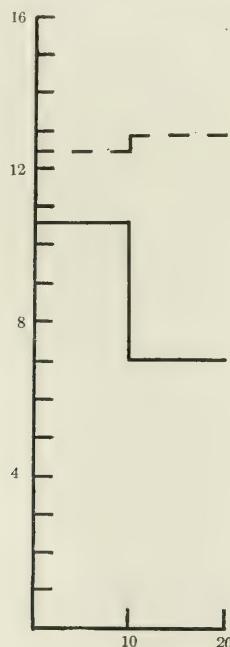


Fig. 13 Polygon of the average number of generations per line per ten-day period produced by the fast and slow sets of lines of Experiment 1-E (balanced selection after the reversed selection of Experiment 1-D). The continuous line is the curve of the reversed selected former fast lines now balanced selected. The broken line is the curve of the reversed selected former slow lines now balanced selected. The ordinates are the averages per ten-day periods; the abscissae the ten-day periods.

the variations in fission rate within the clone. For one hundred and thirty days two halves of this clone were subjected to selection in opposite directions; this produced a marked and steadily increasing difference in the average fission rate of the two halves. Expressing the excess of generations produced by the fast-selected lines as a percentage of the total number of

TABLE 11  
*Experiment 1-E; Actual number of generations per 10-day period per line. Balanced selection*

LINE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	STRENGTHS SUBMISSION NUMBER	AVERAGE AGE
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL	STRENGTHS SUBMISSION NUMBER	AVERAGE AGE
First 10-day Period:																																	
Fast.....	11	11	15	11	13	9	11	11	14	12	16	16	15	15	13	9	11	14	14	14	13	13	9	13	12	13	12	13	372	0	12.40		
Slow.....	11	11	11	11	11	10	10	10	10	10	11	11	11	11	11	10	9	9	11	11	11	11	11	10	10	9	317	0	10.56				
Excess in favor of the fast selected lines.....	0	0	4	0	2	-1	1	1	4	2	5	5	4	4	2	-2	1	4	5	5	5	2	-2	2	-2	3	2	4	1.84				
Second 10-day Period:																																	
Fast.....	14	13	10	13	17	13	14	15	11	11	10	10	11	11	15	16	16	21	11	20	12	12	14	9	10	15	14	9	11	9	387	0	12.86
Slow.....	5	7	8	7	8	6	7	8	9	8	5	6	8	7	7	5	9	10	6	6	9	9	7	7	5	8	9	6	8	2	212	0	7.06
Excess in favor of the fast selected lines.....	9	6	2	6	9	7	7	2	3	5	4	3	4	8	11	7	11	5	14	3	3	7	2	5	7	5	3	3	7	580			

TABLE 12  
*Experiment 1, Parts I, 2, 3 and 4: Total number of generations produced by each line, fast and slow during the 13 10-day periods of Experiment 1*

LINE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	TOTAL
Fast.....	183	183	183	184	181	181	186	178	182	181	180	185	186	183	185	187	185	186	184	181	185	180	183	179	180	181	187	181	185	5506	
Slow.....	124	125	127	126	128	128	127	122	124	126	125	125	122	128	125	117	123	122	120	123	118	119	119	118	118	119	119	119	119	120	3678
Excess in favor of the fast lines.....	61	58	58	57	56	53	54	64	54	56	55	63	58	68	64	63	63	64	61	67	61	61	66	71	68	65	65	65	65	1828	
Range of Fast lines.....	178	187																													
Range of Slow lines.....	116	128																													

generations produced by both sets, the difference was 6.9 per cent in part 1; 12.8 per cent in part 2; 19.3 per cent in part 3, and 21.2 per cent in part 4. Table 12 summarizes the generations produced by each line of each set throughout all parts of the experiment. It shows that for the fast lines the number of generations ranges from 178 to 187, while for the slow lines the range is but from 116 to 128. Thus there is no overlapping in the two sets; the slowest fast selected line has produced 50 more generations than the fastest slow selected line.

To determine whether the difference in fission rate thus produced is heritable, parts of the two sets were removed at intervals and subjected to culture without selection ('balanced selection'). In every case it was found that the difference was heritable. Also, representatives of the two sets after eighty days of selection and forty days of no selection, were subjected to mass culture for twelve days. Further line culture for fifty days without selection showed that the inherited difference in fission rate still persisted. Thus the inherited difference produced by selection had lasted for one hundred and two days without selection.

Experiments with reversed selection showed that the inherited difference could be reversed as readily as it is produced; the originally fast set was thus caused to become the slower one, and vice versa. Continuation of these sets without selection showed again that the difference so produced was heritable.

Thus in this case the selection of small individual variations in fission rate has split the single clone (derived vegetatively from a single parent) into two hereditarily diverse divisions (diverse clones).

## *2. The second series of experiments*

The results of the first series of experiments appeared so important and in some respects unexpected that it was felt necessary to control them by repetition, beginning again with a single individual, and endeavoring anew to procure from it by selection two hereditarily diverse sets. This was first at-

tempted with a single individual taken from one of the fast lines of the first set of experiments, giving experiment 2, described below. It was later carried out anew with the progeny of a single 'wild' individual ('third series').

*Experiment 2-A.* Selection among the progeny of a single individual from Experiment 1. The individual selected for repetition of the experiment was one of those belonging to a fast line of the previous experiment. The progeny of this individual did not live well, so that in some cases one or both sets died out before any definite result was obtained. In one case, however, selection of fast and slow sets was continued through nine consecutive ten-day periods (April 5 to July 3, 1914). During every ten-day period except the first set the fast-selected set produced more generations than the slow-selected one. The results are less striking than in Experiment 1, however, in the fact that on twenty-two days out of the ninety, the slow lines produced more generations than the fast ones. The irregularity appears connected with the high mortality in both sets. However, the average difference in fission rate per line per day in favor of the fast selected set was 0.317 for the first thirty days, 0.757 for the second thirty days; and 0.61 for the third thirty days.

*Experiment 2-B.* Now the two sets resulting from the ninety days' selection in Experiment 2-A were subjected to balanced selection for ten days. The difference in fission rate persisted to the extent of an average difference in favor of the fast lines of 0.28 generation per line per day, though on one day the slow lines were faster by 0.01 generation per line. Before the end of the next period all lines were dead, owing perhaps to the hot weather.

*Results.* The evidence from these two experiments is, so far as it goes, in the same direction as from those of the first set. Selection produced from the progeny of a single individual two sets differing hereditarily in average fission rate.

*3. The third series of experiments*

*Experiment 3-A.* September 22 to October 21, 1914.

To further test the results thus far reached, and to determine whether they are based on conditions generally occurring in the organism studied, a new wild individual was obtained from a new mass culture brought into the laboratory September 15, 1914. From this, two sets of thirty individuals each, all belonging to the seventh filial generation, were obtained, and sub-

TABLE 13

*Experiment 3-A: Actual number of generations and of selections per ten-day period per thirty lines of the 30 fast and 30 slow lines isolated among the progeny of the single 'wild' individual subjected to opposite selections for 30 days*

TEN-DAY PERIODS	FIRST	SECOND	THIRD	TOTAL	AVERAGE PER LINE
<b>Fast Lines:</b>					
Average number of selections.	2.53	1.60	1.50		
Total number of generations.	973	634	603	2210	73.66
Average number of generations.....	32.43	21.13	20.10		
<b>Slow Lines:</b>					
Average number of selections.	2.90	1.53	0.96		
Total number of generations.	935	581	523	2039	67.96
Average number of generations.....	31.16	19.36	17.43		
Actual excess in favor of the fast lines.....	38	53	80	171	5.70
Average excess in favor of the fast lines.....	1.27	1.77	2.67		1.90
Percent the difference is of the total for both.....	1.99%	4.36%	7.10%		

jected, in the manner previously described, one to 'fast,' the other to 'slow' selection. In this series only one individual, in place of two, was selected from each line at each change, and the selections were made daily. There was no reduplication of the fastest and slowest lines. Fourteen of the fast and twenty-one of the slow persisted intact throughout experiments 3-A and 3-B.

Opposite selection was practised for thirty days. The records are given in table 13. In all three ten-day periods the fast-selected lines produce more generations than the slow-selected,

though in four days of the first period the reverse is true. In all the other twenty-six days the average of the fast lines was above that of the slow. The table shows a gradual increase in the number of generations produced by the fast lines relative to those produced by the slow, the excess in favor of the fast



Fig. 14-a Polygon of the average number of generations per line per three day period produced by the fast and slow sets of lines of Experiment 3-A (direct selection in opposite directions of the progeny of the second wild individual). The continuous line is the curve of the fast set, the broken line, the curve of the slow set. The ordinates are the averages, the abscissae the three-day periods.

Fig. 14-b Curve of the difference (in favor of the fast lines) between the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-A (direct selection). The ordinates are the differences between the averages; the abscissae, the consecutive three-day periods. Note the progressive increase of the difference under opposite selection.

being for the three successive ten-day periods respectively 1.99 per cent, 4.36 per cent, and 7.10 per cent of the total number of generations produced in the given period.

The gradual increase of the difference between the two sets indicates that this difference was heritable. This gradual increase is well shown in the curves of figure 14, giving at *a*, the average number of generations produced per three-day period by each set, at *b* the curve of the differences in favor of the fast set. Figure 15 gives the curves of variation of the total number of generations produced by the two sets, showing that they overlap very little. The evidence indicates strongly that the effect of selection is cumulative.

*Experiment 3-B.* To test whether the difference produced by selection is actually heritable, balanced selection was now practiced for twenty-one days, October 21 to November 10, 1914. On every day but one (the second) the lines that had been subjected to fast-selection averaged higher than others. Table 14 gives the actual numbers of generations per line for the two ten-day periods.

Table 14 shows that during both of its periods the fast lines averaged more generations than the slow ones and further that the per cent that this difference is of the total number of generations produced by both together was practically constant. Figures 14-*c* and 14-*d* show the average difference of fission rate between these two sets of lines, averaged for three-day periods. These two figures emphasize the marked uniformity of this average difference. Hence this experiment shows that the opposite selection previously practised had produced a nearly uniform heritable difference of average fission rate between the two halves of this second clone. The results are thus the same as in our first set of experiments.

*Experiment 3-C.* Effects of conjugation on the results of selection.

It appeared of interest to determine whether these inherited results of selection would persist through conjugation. It is well known that conjugation is an ordeal having many effects on

TABLE 14

**Experiment 3-B:** Actual number of generations per ten-day period per line of the lines of Experiment 4-A for the twenty-one days of balanced selection immediately following the thirty days of opposite selection of Experiment 4-A, with the differences in favor of the fast-selected lines. The column marked per cent shows what percentage this difference is of the total produced by both sets

vitality and reproductive power; often changing the fission rate. Conjugation was obtained among the selected individuals of this third set of experiments, and its effect tested.

Watch glass cultures were made in  $\frac{1}{32}$  per cent malted milk from each of the thirty fast lines and the thirty slow lines. On

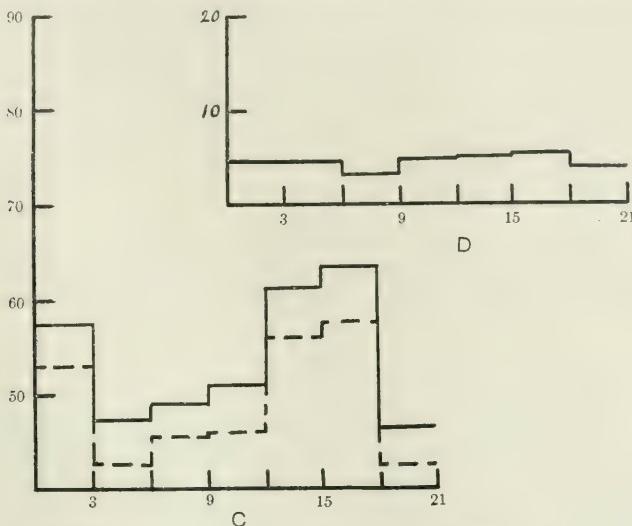


Fig. 14-c Polygon of the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-B (balanced selections after the direct selection of Experiment 3-A). The continuous line is the curve of the fast set, the broken line is the curve of the slow set. The ordinates are the averages, the abscissae the three-day periods.

Fig. 14-d Curve of the difference (in favor of the fast lines) between the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-B (balanced selection after the direct selection of Experiment 3-A). The ordinates are the differences between the averages; the abscissae, the consecutive three-day periods. Note the practical uniformity of the difference under balanced selection.

December 4 and succeeding days conjugating pairs were obtained, some from the fast lines, some from the slow ones. Though many of the ex-conjugants died, eventually representatives propagating normally were obtained from six of the fast lines and four of the slow ones of Experiment 3-B.

It will be recalled that these animals, before conjugation, had been subjected to opposite selection for thirty days, then to

twenty-one days of culture without selection and twenty-three days of mass culture. On December 7, sixty ex-conjugants were isolated from the fast lines (ten from each of six diverse lines), and sixty from the slow lines (twenty from each of two slow lines, ten from each of two others). They were cultivated in the way previously described. Balanced selection (i.e., in effect no selection) was practised for fifteen days. Table 15 gives the numbers of generations produced by the fast and slow lines during the five three-day periods of this experiment. No lines were lost by death, and practically no selection of any sort was necessary.

The daily record sheets of this experiment show that during its first five days the slow lines averaged more generations per line than did the fast lines. Table 15 shows this for the first

TABLE 15

*Experiment 3-C: Actual number of generations of the 60 fast lines of ex-conjugants and of the 60 slow lines of ex-conjugants during balanced selection for 15 days. The two members of each conjugating pair were individuals of the same line*

THREE-DAY PERIODS	FIRST	SECOND	THIRD	FOURTH	FIFTH	TOTAL	AVERAGE PER LINE
<b>Fast lines:</b>							
Total number of generations.....	378	302	188	237	192	1297	21.63
Average number of generations.....	6.30	5.03	3.13	3.95	3.20		
<b>Slow lines:</b>							
Total number of generations.....	416	310	178	225	181	1310	21.83
Average number of generations.....	6.93	5.16	2.96	3.75	3.02		
Actual excess in favor of the fast lines.....	-38	-8	10	12	11	-13	-0.20
Average excess in favor of the fast lines.....	-0.63	-0.13	0.17	0.20	0.18		-0.04
Per cent of the excess in favor of the fast lines in terms of the total for both.....	-4.78%	-1.30%	2.73%	2.60%	2.94%		

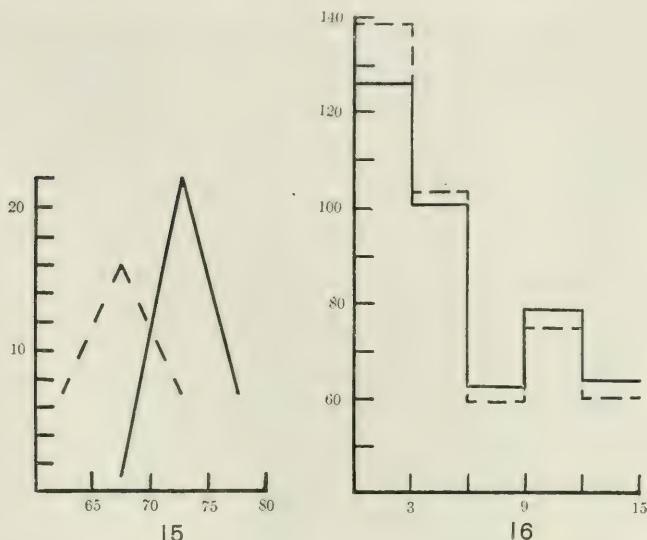


Fig. 15 Curves of variation of the lines of Experiment 3-A (direct selection of progeny of second wild individual). The ordinates give numbers of lines; the abscissae the number of generations. The continuous line is the curve of the fast set; the broken line, the curve of the slow set.

Fig. 16 Polygon of the average number of generations per line per three-day period produced by the fast and slow sets of lines of Experiment 3-C (balanced selection after conjugation). The continuous line is the curve of the fast set, the broken line, the curve of the slow set. The ordinates are the averages; the abscissae, the three-day periods.

two three-day periods and also that there is a marked decrease in both the actual and percentage difference. During the last three of the three-day periods the difference was uniformly in favor of the sixty fast lines and was remarkably constant. Figure 16, the polygon of the average number of generations produced by each set of sixty lines per day during the consecutive three-day periods of this experiment, represents this same result graphically.

Figure 17-a and 17-b give the curves of variation from the diverse lines, the former for the first six days, the latter for the last nine days. Why the ex-conjugants of the fast selected lines should for the first five days be slower than the ex-conjugants of the slow selected ones is not clear. But this condition existed

only during the period of reorganization after conjugation, when the fission rate in all was extremely low. As soon as the 'normal' fission rate was resumed, the balanced-selected set of origi-

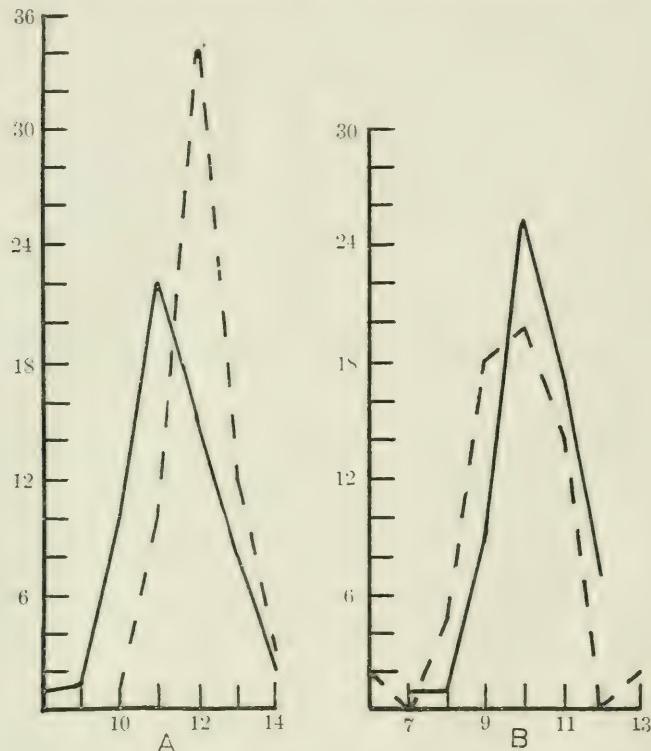


Fig. 17-a Curves of variation of the lines of Experiment 3-C (balanced selection after conjugation) for the first 6 days of the experiment. The continuous line is the curve of the fast set; the broken line, the curve of the slow set. The ordinates are the numbers of lines; the abscissae, the number of generations.

Fig. 17-b Curves of variation of the lines of Experiment 3-C (balanced selection after conjugation). The continuous line is the curve of the fast set; the broken line, the curve of the slow set. The ordinates are numbers of lines; the abscissae, generations.

nally fast ex-conjugants at once resumed its place in advance of the balanced-selected set of originally slow ex-conjugants and continued to divide more rapidly throughout the rest of the experiment.

Thus it is clear that the heritable difference in fission rate brought about by selection during vegetative reproduction is not lost when the animals conjugate, but persists through that ordeal.

*Statement of the general results of the third series of experiments.* This third series of experiments has entirely corroborated the results of the first and second series. In a second clone, entirely unrelated to that used for the first and second series of experiments, opposite selection for thirty days produced a heritable difference of average fission rate, a difference that gradually increased as selection progressed, indicating again that the effect of selection on this physiological character is cumulative. This average difference persisted through twenty-one days of balanced selection, twenty-nine days of mass culture followed by conjugation and then fifteen days of further balanced selection.

#### DISCUSSION AND CONCLUSIONS

All the experiments thus give concordant results; through selection of individual differences in fission rate it is possible to divide a clone into two divisions differing hereditarily in rate of multiplication. The effects of selection are cumulative; the hereditary differences between the two divisions become greater the longer selection continues. By reversing the direction of selection the hereditary differences between the sets are reversed.

Is this effect of selection due to the slow accumulation of small variations, or to the chance isolation of mutants differing markedly from the type? The whole character of the results indicates strongly that the former is the case, and this indication is borne out by careful study of the records. There is no sudden change at a definite point, indicating the appearance of a mutant. The steady cumulative effect of continued selection can not be explained on the mutant theory without giving such a meaning to the word mutant as removes any distinction between it and 'slight individual variation.' It would require us to assume the repeated appearance of successively faster and faster mutants in each of the thirty fast selected lines, of successively slower and slower mutants in each of the thirty slow-selected lines; a conception which coincides with the view that selection operates cumulatively on slight individual variations.

Woodruff and Erdmann ('14) show that in *Paramecium* during vegetative reproduction there are periodical reorganizations of the nucleus, the vegetative macronucleus being replaced by a new portion of the reserve micronucleus. It has been suggested that this new macronucleus may give an altered hereditary constitution, so that at each reorganization inherited variations may appear. Are the effects of selection herein described based on such variations occurring thus at the time of reorganization?

The relatively short time required for producing inherited differences among the progeny of a single individual make it improbable that the variations in *Stylonychia* are to be accounted for in this way. As we have seen, marked differences appear after ten days of selection, and these are gradually increased in the next ten days, and again in the next, and so on. The percentage of difference in proportion to the total average fission rate for the 13 consecutive ten-day periods of Experiment 1 are 5.41, 10.35, 5.58, 7.62, 23.81, 13.69, 19.20, 21.93, 15.19, 24.71, 26.05, 25.35 and 19.23. These percentages for the three consecutive ten-day periods of Experiment 3 are: 1.99, 4.36 and 7.10. Now in *Paramecium* the interval between reorganizations is about thirty days. If in *Stylonychia* the interval is of about this length, it would be quite impossible to account on this ground for the cumulative effects of selection occurring within periods much shorter; selection should show sudden effects immediately after the reorganization in a given stock, and should then be quite without effect during the intervening periods. Nothing of this sort appears in the records of the present experiments.

The main interest of this particular matter lies in its bearing on the question whether variations are definite and limited in extent and possible number, as in rigid Mendelian recombinations of invariable factors; or whether variations may be of indefinitely many diverse extents and are not limited by a precise numerically definable factorial structure of the germinal material. If the nuclear reorganization described by Woodruff and Erdmann takes place in a definite way, comparable to the known reductions and recombinations in the chromosomal apparatus at the formation of the germ cells, then this could not be

a source of indefinite and unlimited variation. After a time the possible combinations of factors would be exhausted, and such constancy would result as Johannsen claims to have found in his 'pure lines' of beans. Evolution thus could not make extended and continuous progress in this way. If on the other hand the nuclear reorganization occurs in no precisely definable way, but with indefinite and unlimited variations, then this apparatus shows precisely the characteristics held to be common to organisms by those who believe in continued evolutionary progress through the accumulation of such indefinite and unlimited variations. Some material basis for such variations would have to be assumed; the nucleus might furnish this as well as any other portion of the organism. But, as we have seen, the present evidence does not favor the idea that the hereditary variations in *Styloonychia* are dependent at all on these nuclear reorganizations.

Our main result, that during vegetative reproduction among the progeny of a single individual selection of small variations produces cumulative hereditary effects, is in marked contrast with the results of most investigators, who, following Johannsen ('03, '09, '11), have found that 'pure lines' or 'clones' are hereditarily constant under selection. Johannsen's results were obtained with self-fertilized lines of beans. Similar ineffectiveness of selection has been found by Hanel ('08) and Lashley ('15) as to the number of tentacles in *Hydra* multiply by budding, by Jennings ('08, '09, '10) for size in infusoria; by Barber ('07) (in the main), and by Winslow and Walker ('09), in bacteria; by East ('10) in the vegetative reproduction of the potato, by Agar ('13 and '14) in Cladocera and aphids multiplying parthenogenetically; and by various other investigators on diverse organisms. Some discordant results have been recorded, but most of these are ill-defined or uncertain; it is mainly in bacteria, with their immense difficulties for precise technique in pedigree work, that heritable variations or modifications have been described. The immense preponderance of evidence has been that in uniparental reproduction heritable variations do not occur (save as rare mutations of marked character), and that selection of slight

individual variations is without effect in altering the hereditary characteristics.

How are we to account for the discrepancy between the present results, and those just mentioned? In *Styloynchia* we are dealing with an organism which is large enough to be easily handled and followed individually, so that no question can arise as to the purity of the pedigrees (as sometimes occurs with reference to *Bacteria*). In this organism the facts as to the cumulative effect of selection are clear.

We are of course dealing with a delicate physiological characteristic, and this is perhaps more readily varied (even hereditarily) than the characters examined by most other investigators. Further, it is perhaps true that hereditary changes are more easily brought about in the *Protozoa* than in the more complex organisms, for in *Protozoa* the 'apparatus of heredity' is in close chemical contact with all the somatoplasm.

But a certain feature of the experimental procedure in the present case may have more importance than these conjectural considerations. It has been possible in my work to make a much greater number of actual selections (where plus and minus cases were both present to choose from), than in most of the work that has given negative results. And it has been found that few selections give very slight results, and that a great number are required to give any marked differences between the sets. Thus, in my main experiment, on the average 39.86 plus selections were made in the fast-selected lines; 34.36 minus selections in the slow-selected lines. The difference between the two sets was thus the equivalent of some 74 selections extending through an average of 150 generations. This resulted in the production of a constant average difference per line of 0.42 of one fission per day.

Contrast with this great number of selections the *six* made by Johannsen in obtaining his negative results with beans, the three or four made by East with potatoes, the two made by Winslow and Walker with bacteria, and similar small numbers made by most other investigators along these lines; even indeed the selection through fifteen generations made by Agar, in

Cladocera. It appears not at all inconceivable that in these organisms an equal number of selections, covering as great a number of generations as were made in *Styloonychia*, would have given similar heritable effects. What all the work shows (and here my own is not in positive disagreement) is that heritable variations of considerable extent do not occur so frequently as was at one time supposed, so that a few selections are not sufficient for establishing a definite positive effect. But negative results from a few selections are not sufficient for disproving the occurrence of heritable small variations which may be gradually accumulated. This indeed has been admitted by many of those that have obtained negative results; thus Johannsen remarked that "there is the possibility that a selection of fluctuating variants, during very many generations, might divert the type of a line" ('03, p. 62); Jennings says "what the pure line work shows (agreeing in this with other lines of evidence) is that the changes on which selection may act are few and far between instead of abundant . . ." ('10, p. 144), and East states that "as a result of these experiments I would not go so far as to say that variations in power of resisting physiological or fungus diseases do not occur in asexual reproduction, but I do believe that the relative possibility that the commercial grower will obtain disease-resisting varieties in this way is negligible" ('10, p. 134).

As a result of this work upon *Styloonychia* it is possible to substitute for such indefinite remarks, precise data as to the occurrence of heritable variations and their accumulation through selection, when sufficiently long continued. And this can hardly fail to have influence on the conception of the hereditary constitution or genotype as a fixed thing, changing only discontinuously by marked steps or mutations, that do not intergrade.

#### SUMMARY

In *Styloonychia pustulata*, by the opposite selection through more than 150 generations of small individual variations occurring among the progeny of a single individual, it was possible to produce two sets differing hereditarily in rate of fission. During selection there was a gradual increase in the average heritable

difference between the two sets, showing that the effect of selection was cumulative.

This result was, at various intervals, subjected to the most rigid tests possible, by balanced selection throughout long periods; by mass culture without selection, and by reversed selection. In every case the results were corroborated. The hereditary differences induced continued through periods of balanced selection lasting longer than the periods of direct selection by which they were induced; they did not disappear save under the effects of reversed selection.

These results were first reached with the progeny of a single individual multiplying asexually. They were then confirmed by beginning anew with a single individual from among this set and obtaining the same results among its progeny. A third set, derived from a wild individual quite unrelated to the first two series, gave the same results. In this third series conjugation occurred within each of the diverse sets produced through selection, and it was found that the hereditary differences persisted through and after conjugation.

Thus in *Styloynchia*, from a single clone of given genotype it is possible to obtain through long continued selection during reproduction by fission, two sets (clones?) of diverse genotype, differing characteristically from each other in rate of fission, under identical conditions; and retaining these differences from generation to generation. The selection of small variations, such as appear within the 'pure strain' or clone, is then an effective evolutionary procedure.

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## VARIATION IN HEAD LENGTH OF SPERMATOZOA IN SEVEN ADDITIONAL SPECIES OF INSECTS<sup>1</sup>

CHARLES ZELENY AND C. T. SENAY

EIGHT FIGURES

A study of variation in head length among spermatozoa from single testes in fifteen species of animals from widely separated groups was made by the senior author and E. C. Faust ('15 a and b). The frequency distribution of the size groups in a great majority of the cases showed bimodality of such a character as to make it highly probable that in these species the spermatozoa are dimorphic as regards size. Furthermore it was shown to be highly probable that this dimorphism is the result of chromosomal differences. There is a close agreement between the ratio in the two groups as calculated on this hypothesis and the actual ratio determined by measurement.

These chromosomal differences as has now been abundantly demonstrated are related to sex determination and the size groups must be similarly related, fertilization of the eggs by spermatozoa of the upper group yielding females and by those of the lower group, males. A probability is thus opened for controlling sex as soon as living spermatozoa of the two sizes can be experimentally separated.

In view of the importance of the question of existence of two size groups and in further preparation for the experimental tests, measurements were made for seven additional species and these are described in the present paper.

Together with those formerly described twenty-two species are now available for drawing a general conclusion. This number includes all that have been measured, with two exceptions. These two are *Helodrilus*, a hermaphroditic form, and the

<sup>1</sup>Contribution from the Zoological Laboratory of the University of Illinois No. 49.

Plymouth Rock Fowl concerning which there is a controversy on the cytological side. They are not included because they have a special interest not connected with our main hypothesis and because it is desirable to make at least one more series of measurements in each species before publishing the data. Emphasis is laid on the fact that all sets of measurements are published, because bimodal distributions may appear occasionally as a matter of chance in a uniform population. The frequency of their occurrence is therefore all-important.

The details of preparation of material and the method of measurement are in all respects similar to those described by Zeleny and Faust ('15 a) except that fixation was in all cases in osmic fumes and staining in haematoxylin. The authors are indebted to Mr. C. A. Hart for identification of the species.

#### DATA

1. *Corizus lateralis*, a hemipteran. The material was obtained at the end of March and gave an abundance of active spermatozoa. Two sets of measurements, each of 500 spermatozoa, were made. As shown in figures 1 and 2 the two determinations agree closely. Each shows a pronounced bimodal curve with modes at  $27.1\ \mu$  and  $29.5\ \mu$ , giving a ratio of  $1.00 : 1.09$ . The intermodal depression is deep and wide and the two elements of the curve are approximately equal as regards number of individuals. There seems to be no doubt of the existence of two distinct size groups with equal numbers of spermatozoa.

The chromosomal history was worked out by Montgomery ('06). He describes two kinds of spermatids, one with six and the other with seven chromosomes. Two size groups are therefore to be expected but the drawings given by Montgomery are not large enough to enable one to make a calculation of the chromatin ratio. In *Anasa tristis*, another member of the Family Coreidae, the expected ratio is  $1.00 : 1.11$ .

2. *Leptocoris trivittatus*, a hemipteran. The material was obtained in early March and gave an abundance of active spermatozoa. Nine hundred and eighty-four measurements were made. The frequency distribution as shown in figure 3 is somewhat irregular but there are two principal modes, one at  $25.4\ \mu$

and the other at  $27.8 \mu$ , giving a ratio of 1.00 : 1.09. The majority of the individuals are grouped around the higher mode. If these modes can be considered as related to the sex chromosomes the

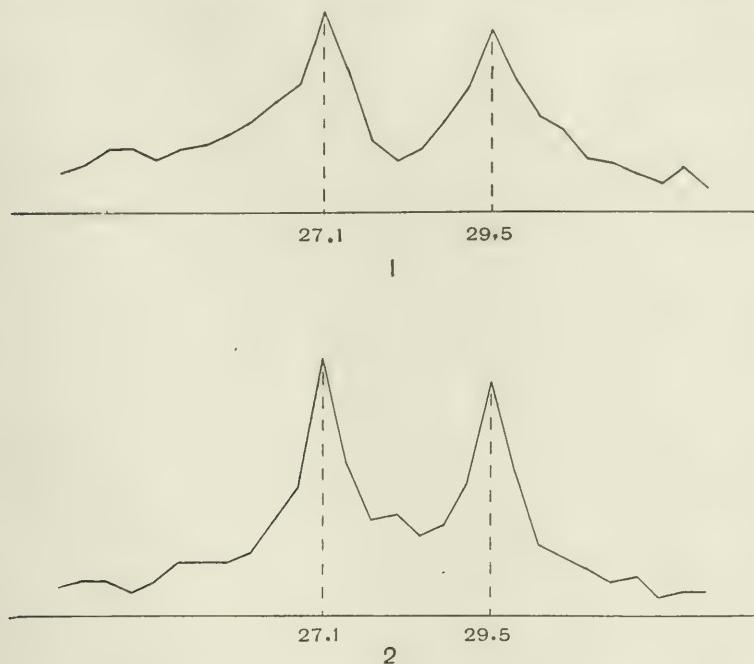
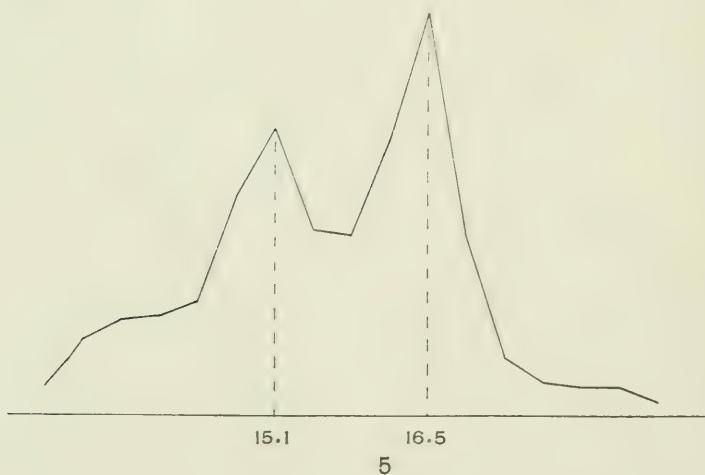
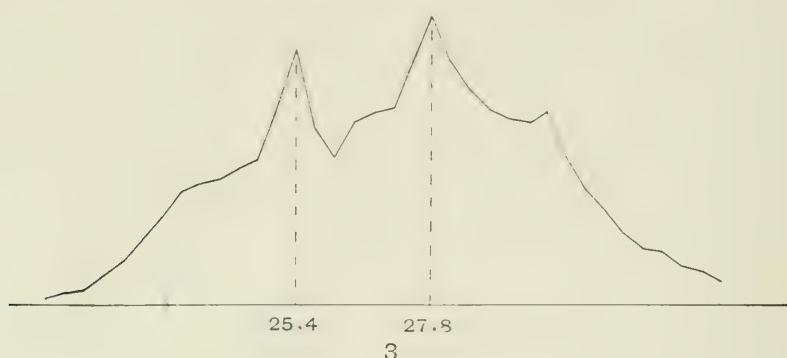


Fig. 1 *Corizus lateralis*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in.....	23.0	23.3	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1
Frequency.....	7	8	10	13	13	11	13	14	16	19
	26.4	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	23	27	42	30	15	11	13	19	26	38
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	
	28	20	17	11	10	8	6	9	5	

Fig. 2 *Corizus lateralis*; frequency distribution of head-lengths of another sample of 500 spermatozoa from the same testis as those given in figure 1.

Value in.....	23.0	23.3	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1
Frequency.....	4	6	7	7	5	7	11	11	11	13
	26.4	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	20	27	54	32	20	21	17	19	28	45
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	
	30	15	12	10	7	8	4	5	5	



group containing the female-determining spermatozoa exceeds the other in numbers. On the basis of the cytological evidence we should expect the two groups to be equal but there is a great deal of evidence in both cytological and experimental data to indicate that one kind is often more numerous than the other.

E. B. Wilson ('06) has described two kinds of spermatids for this species, one containing six and the other seven chromosomes. In the absence of figures it is not possible to determine an expected ratio. Since this species also is a Coreid, the ratio of 1.00 : 1.11 determined from the chromosomes of *Anasa* is of interest. It agrees fairly closely with the measurements of head-lengths in this as well as in the last species.

3. *Reduviolus ferus*, a hemipteran. The material was collected during March and gave active spermatozoa. The results of the five hundred measurements are given in figure 4. The fre-

Fig. 3 *Leptocoris trivittatus*; frequency distribution of head-lengths of 984 spermatozoa from a single testis.

Value in.....	20.9	21.3	21.6	22.0	22.3	22.7	23.0	23.3	23.7
Frequency.....	1	2	3	6	9	13	18	23	25
	24.0	24.4	24.7	25.0	25.4	25.7	26.1	26.4	26.7
	26	28	30	41	53	37	31	38	40
	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5	29.8
	41	50	60	51	45	41	39	38	40
	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6	33.0
	31	24	20	15	12	11	8	7	5

Fig. 4 *Reduviolus ferus*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in.....	23.7	24.0	24.4	24.7	25.0	25.4	25.7	26.1	26.4
Frequency.....	4	5	6	5	12	19	25	30	35
	26.7	27.1	27.4	27.8	28.1	28.4	28.8	29.1	29.5
	36	49	56	45	33	27	35	22	18
	29.8	30.1	30.5	30.9	31.2	31.6	31.9	32.3	32.6
	18	10	9	8	7	5	4	2	1

Fig. 5 *Euschistus variolarius*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns.....	13.0	13.4	13.7	14.1	14.4	14.7	15.1	15.5	15.8
Frequency.....	6	16	20	21	24	46	60	39	38
	16.1	16.5	16.8	17.2	17.5	17.9	18.2	18.5	
	58	84	38	12	7	6	6	3	

quency distribution gives a major mode at  $27.4 \mu$  and a minor one at  $28.8 \mu$ . The minor mode may be accidental though such an irregularity is not common in frequency distributions in a population known to be homogeneous.

There are no data available concerning the spermatogenesis of this species but Montgomery ('06) has described two kinds of spermatids for other members of the family.

4. *Euschistus variolarius*, a hemipteran. Material was obtained during April. All the spermatozoa were not fully developed and selection was necessary to insure the exclusion of the unripe ones. Five hundred measurements were made. The resulting curve as shown in figure 5 is distinctly bimodal with modes at  $15.1 \mu$  and  $16.5 \mu$  and with an inequality favoring the larger spermatozoa. The ratio between the modes is  $1.00 : 1.09$ .

E. B. Wilson ('06) described an "X" and a "Y" chromosome for this species. The expected ratio as approximated from his figures of the chromosomes is  $1.00 : 1.04$ . This does not agree at all well with the ratio between the modes as given above. This result is contrary to that obtained for several species by Zeleny and Faust ('15) which showed a striking similarity between the two calculations.

5. *Cosmopepla carnisfex*, a hemipteran. The material was obtained in May and the spermatozoa were all active. Five hundred measurements were made. The resulting curve as given in figure 6 shows well marked bimodality with approximate equality in the two groups. The modes are at  $18.5 \mu$  and  $19.9 \mu$  and give a ratio of  $1.00 : 1.075$ . There can be no doubt in this case of the existence of two fairly equal size groups.

Montgomery ('06) describes two kinds of spermatids for this species, one with seven chromosomes plus an "X" and the other with seven plus a "Y." The figures are not suitable for the determination of the chromatin ratio. The ratio in another Pentatomid, *Euschistus variolarius*, is  $1.00 : 1.04$ .

6. *Passalus cornutus*, a coleopteran. Material was obtained at the end of March and the spermatozoa were uniformly ripe and active. Five hundred spermatozoa were measured. Neglecting the minor projection at the right, the curve as given in figure

7 is unimodal with the mode at  $11.7 \mu$ . This indicates either a single group or two so close together as to give a unimodal result (see Zeleny and Faust '15 a, p. 193).

There are no published spermatogenesis data for this species. Descriptions for different species of beetles show in all cases, except one, two groups as regards chromatin content. Leaving out the possibilities of random sampling and of selective elimination within the two groups the present species seems to be either without distinction among its spermatozoa or else has two groups differing but slightly from each other.

7. *Berosus striatus*, a coleopteran. The material was obtained in early April and the spermatozoa were all ripe and active. The frequency distribution of the five hundred measurements is given in figure 8. The curve is distinctly bimodal with approximate equality in the two groups. The modes are at  $16.1 \mu$  and  $17.2 \mu$  with a ratio of  $1.00 : 1.07$ .

There are no cytological data for this species but as was stated in discussing the last form the descriptions for all but one species of beetles give two kinds of chromosome groups among the spermatids.

#### DISCUSSION

The present data as a whole substantiate the view that dimorphism in size of spermatozoa is of common occurrence in those groups of animals in which two chromosomal classes of spermatids are of common occurrence. Of the seven species described five are hemipterans and two coleopterans. In both of these orders a great majority of the species so far studied show a quantitative difference in chromosomal content among the spermatids though in each case there are some species which show no difference or only a slight one.

Among the five hemipterans three, *Corizus lateralis*, *Euschistus variolarius* and *Cosmopepla carnicifex*, show two well marked size groups. The other two species are not so clear. One, *Leptocoris trivittatus*, is probably dimorphic though the curve is irregular. The other, *Reduviolus ferus*, is doubtful because the upper mode is very low and may be a chance projection in

a unimodal distribution. It should not, however, be forgotten that the two elements of a population will, when combined, give a unimodal curve in case the modes of the elements are close together.

Among the coleopterans one, *Berosus striatus*, has two well marked and equal size groups. The other, *Passalus cornutus*, has a single size group or two with modes very close together.

Taking all cases so far described there are twenty-three species involved, including the one given by Wodsedalek ('13). This is a sufficient sample to justify us in stating that there can be no doubt of the validity of the hypothesis presented. The chromosomal dimorphism of spermatogenesis is represented in the active functional spermatozoa by size dimorphism. Control of sex then merely awaits our ability to separate the two sizes in the living condition and to use them in artificial insemination.

Fig. 6 *Cosmopepla carnifex*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

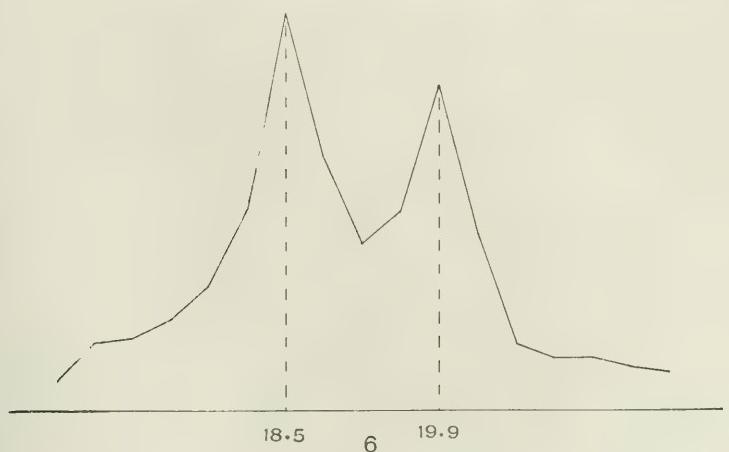
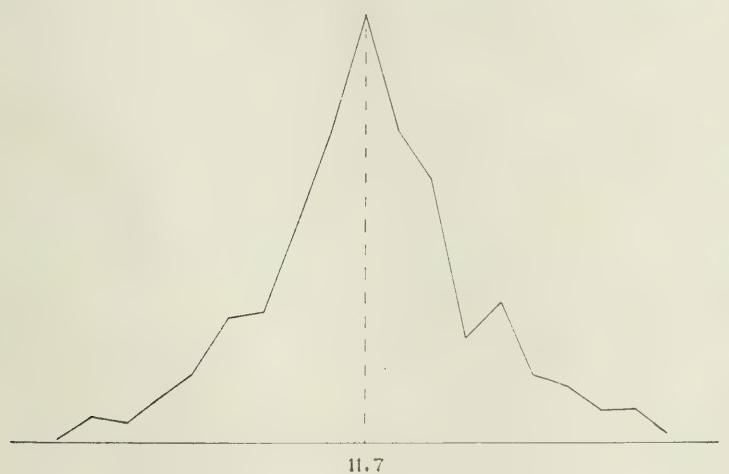
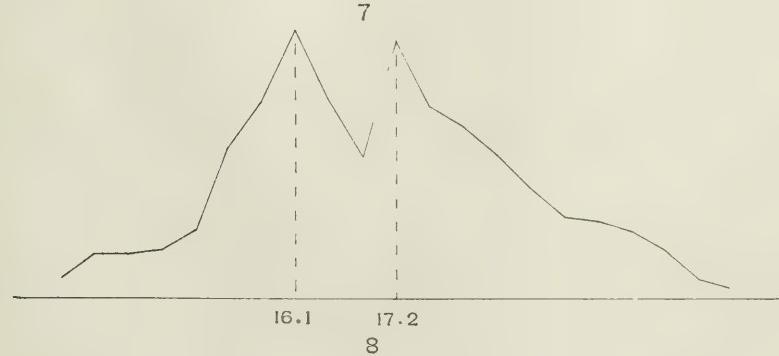
Value in microns.....	16.5	16.8	17.2	17.5	17.9	18.2	18.5	18.9	19.2
Frequency.....	6	14	16	19	26	42	83	53	35
	19.6	19.9	20.3	20.6	20.9	21.3	21.6	22.0	
	42	68	37	14	11	11	9	8	

Fig. 7 *Passalus cornutus*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns.....	10.15	10.30	10.45	10.60	10.80	11.00	11.15
Frequency.....	1	5	4	9	14	26	27
	11.30	11.50	11.70	11.85	12.00	12.20	12.40
	46	65	89	65	55	22	29
	12.55	12.70	12.85	13.00	13.20		
	14	12	7	7	2		

Fig. 8 *Berosus striatus*; frequency distribution of head-lengths of 500 spermatozoa from a single testis.

Value in microns.....	13.7	14.1	14.4	14.7	15.1	15.5	15.8
Frequency.....	4	9	9	10	14	32	41
	16.1	16.5	16.8	17.2	17.5	17.9	18.2
	56	42	30	54	40	36	30
	18.5	18.9	19.2	19.6	19.9	20.3	20.6
	23	17	16	14	10	4	2

18.5      19.9  
611.7  
716.1      17.2  
8

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# THE EFFECT OF SELECTION UPON THE 'BAR EYE' MUTANT OF DROSOPHILA<sup>1</sup>

CHARLES ZELENY AND E. W. MATTOON

FIVE FIGURES

The selection experiment described in the present paper was made with a view to testing the germinal uniformity as regards the distinguishing characteristic in a recent mutant, the 'bar eye' race of *Drosophila*. In this race the ommatidia are reduced in number and the facets are restricted to a vertical band or 'bar' as shown in figure 1. The characteristic appeared in a single male during 1913 (Tice, '14)<sup>2</sup> and the whole 'bar eye' stock is descended from this individual. The race has undergone no apparent change during the two years of its existence. Our material was obtained in January 1914 through the kindness of Prof. T. H. Morgan.

There is a pronounced sexual dimorphism in the number of facets, the males averaging 98.03 and the females 65.06. In every case in the present paper the female number is transformed to the male basis by multiplying it by  $\frac{98.03}{65.06} = 1.51$ .

There is often a slight difference between the number of facets in the right and that in the left eye. For one hundred individuals the average difference was 0.245 per cent in favor of the left eye. This difference is obviously not significant. The right eye only is given in the present records.

The number of facets seems not to vary with the length of the period of development. In five broods counts were made of the facets of the earlier emerging individuals and compared

<sup>1</sup> Contribution from the Zoölogical Laboratory of the University of Illinois No. 50.

<sup>2</sup> Tice, S. A. 1914. A new sex-linked character in *Drosophila*. Biological Bulletin, vol. 26, pp. 221 to 230.

with those of the later emerging ones. The averages for the two are approximately equal.

A sample of five hundred individuals, 250 males and 250 females, taken from the general population of the mutant showed a range of variation in the number of facets from 45 to 182 with

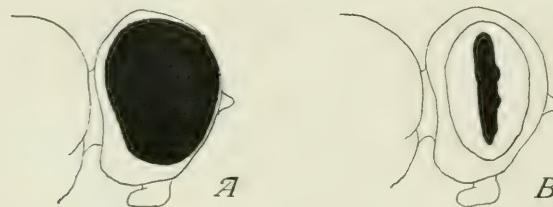


Fig. 1 A. Normal full eye of *Drosophila*. B. 'Bar' eye. The dark areas are the faceted regions.

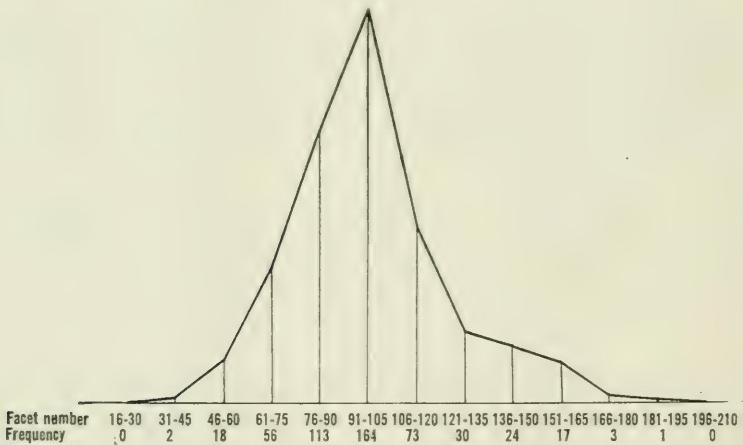


Fig. 2 Variation of facet number in the unselected population of the 'bar' eye mutant of *Drosophila ampelophila*. Five hundred individuals are represented, two hundred and fifty males and two hundred and fifty females reduced to the male level.

a mean of  $98.03 \pm 0.73$  and a standard deviation of  $\pm 24.30$ . The variation curve is shown in figure 2. The average for ten individuals of the normal wild race was 701.1 facets.

Unselected stock was carried as a control through the period of the experiment. There was practically no change in the

number of facets, the average of 250 individuals at the beginning of the experiment being 98.04 and of the same number at the end of the experiment 98.03. There is thus no change in facet number in the absence of selection.

In making the first selection, food containing eggs and larvae was removed from the culture of the 'bar eye' stock and placed in glass vials. Every twelve hours the individuals which had emerged from the pupal cases were slightly etherized and an estimate was made of the number of facets under the low power of the microscope. Males and females with high or low numbers were selected out, high being mated with high and low with low. Each pair was placed in a small bottle with sufficient food to last until all the offspring of the first brood had reached the adult stage. When larvae began to appear in the bottle the parents were killed by etherization and an exact count was made of the facets. As the offspring emerged in the adult form estimates of facet number were again made and high selections were made in the 'high' lines and low selections in the 'low' lines. The final exact facet counts were in all cases made in killed individuals.

The experiment has proceeded far enough so that data are complete for three successive selections in each of three 'high' lines, called A, B, and C, and in each of three 'low' lines, called D, E and F. Fifty individuals, 25 males and 25 females from a single pair, were measured for each generation in each of the lines, with the exception of the third generation in line B where only forty-six were available.

The data for the individual lines are given in tables 1 to 6 and a summary of the six lines is given in table 7. Figures 3, 4 and 5 give in graphic form the course of the selections, each figure combining a 'high' with a 'low' selection line. The mean values, the extreme variates and the mid-parental values are here represented in diagrammatic form for each of the selections.

In all cases there is a significant shifting of the mean as a result of selection. The general population mean of 98.0 is changed in plus line A to 108.7 by the first selection, to 127.5 by the second and to 135.5 by the third. In plus line B the

TABLE 1  
*Plus selection*  
Line A

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	127	133	179	169	177	195
Mid-parental values.....	130		174		186	
Offspring.....	67	69	84	89	92	95
	79	71	88	91	93	97
	86	74	90	94	98	103
	91	76	95	97	98	109
	92	83	95	100	103	115
	92	88	97	103	107	119
	94	89	98	110	108	124
	96	91	99	110	109	127
	99	94	99	112	117	127
	101	100	102	113	117	131
	102	101	103	115	118	131
	103	106	118	119	126	134
	105	106	124	122	126	136
	107	107	125	125	135	137
	107	110	125	133	139	137
	109	115	130	134	140	142
	112	116	133	137	141	143
	116	118	142	139	142	151
	118	128	147	142	154	153
	118	133	148	148	159	157
	122	148	176	153	167	160
	127	149	177	156	180	163
	134	149	180	163	187	171
	140	151	189	187	192	174
	179	169	210	210	204	186
Mean of offspring.....	98.03 ± 0.73	108.7 ± 2.3	127.5 ± 3.1	135.5 ± 2.8		

corresponding figures for the three selections are 110.1, 128.6 and 141.9, and in plus line C, 116.9, 133.5 and 141.0. In minus line D the general population mean of 98.0 is changed to 88.3 by the first selection, to 85.5 by the second and to 81.7 by the third. In minus line E the corresponding figures for the three

TABLE 2  
*Plus selection*  
 Line B

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	139	121	184	165	182	222
Mid-parental values.....	130		174.5		202	
Offspring.....	68	57	86	76	96	89
	74	76	90	79	99	95
	75	80	92	95	100	104
	79	85	93	101	102	116
	80	85	95	103	118	118
	82	89	97	107	121	122
	89	91	99	110	123	124
	90	91	101	111	124	124
	95	95	108	113	128	131
	98	95	118	119	130	136
	98	97	122	127	139	137
	100	100	125	131	141	137
	102	106	126	134	145	148
	104	108	127	137	147	154
	105	118	132	137	154	156
	108	124	137	139	156	159
	112	125	140	143	158	163
	115	128	144	145	160	165
	131	131	144	148	169	166
	133	133	160	149	172	169
	145	137	166	157	175	175
	147	145	171	163	197	189
	156	148	172	175	207	207
	167	149	182	201		
	184	165	183	222		
Mean of offspring.....	98.03 ± 0.73	110.1 ± 2.7	128.6 ± 3.2	141.9 ± 2.9		

selections are 93.9, 89.6 and 84.8 and in minus line F, 94.6, 89.8 and 84.7.

As a result of the three minus and the three plus selections the mean value of the individuals in the plus lines (139.5) is greater than the highest extreme individual of the three minus lines

TABLE 3  
*Plus selection*  
Line C

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	178	157	208	159	194	198
Mid-parental values.....	167.5		183.5		196	
Offspring.....	82	63	67	79	95	97
	85	71	93	88	98	100
	86	85	94	91	100	106
	90	89	97	98	108	107
	93	101	99	101	109	116
	96	103	100	110	118	127
	97	106	101	115	119	127
	99	106	102	118	120	130
	100	107	105	118	120	130
	101	112	105	121	121	131
	103	113	113	122	127	137
	112	121	118	124	134	137
	117	121	133	127	139	139
	118	124	135	130	142	140
	121	128	140	137	143	143
	122	130	140	149	151	146
	123	131	141	153	158	149
	124	133	154	153	162	157
	126	134	160	165	163	162
	126	136	168	174	165	163
	131	142	169	174	166	165
	146	151	177	177	177	172
	147	152	189	180	182	180
	176	159	193	186	187	181
	208	163	194	198	213	205
Mean of offspring.....	98.03 ± 0.73		116.9 ± 2.6	133.5 ± 3.3	141.0 ± 2.8	

(137.0) and the mean of the three minus lines (83.7) is lower than the lowest extreme individual of the three plus lines (89.0).

The coefficients of variability as given in table 7 show on the average a slight decrease in the selected stock as compared with the general unselected population. This difference however can-

TABLE 4  
*Minus selection*  
Line D

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	52	60	69	71	63	58
Mid-parental values.....	56		70		60.5	
Offspring.....	44	56	49	56	48	36
	65	62	54	58	51	50
	67	65	63	59	56	53
	69	66	66	60	58	60
	69	71	69	63	64	60
	70	74	75	69	66	60
	74	74	75	75	68	62
	78	75	76	77	69	65
	79	78	77	79	74	68
	87	82	78	82	74	68
	89	82	78	85	75	71
	90	83	84	86	77	71
	92	85	88	86	79	72
	93	91	89	88	83	74
	95	94	91	91	87	88
	96	98	92	91	88	91
	97	101	92	92	89	95
	97	103	95	92	92	98
	98	103	99	94	97	104
	100	103	100	97	100	104
	102	106	102	100	100	107
	108	109	104	103	103	115
	110	110	105	103	104	115
	115	116	110	104	115	116
	116	127	121	133	137	127
Mean of offspring.....	98.03 ± 0.73	88.3 ± 1.8	85.5 ± 1.8	81.7 ± 2.1		

not be considered as significant because of its irregularity and slight amount.

The progression of the mean is much more rapid in the plus than in the minus lines. The averages for the three plus selections give respectively increases of 13.9, 18.0 and 9.6. The

TABLE 5  
*Minus selection*  
 Line E

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	82	80	64	74	60	62
Mid-parental values.....	81		69		61	
Offspring.....	53	50	50	51	46	57
	64	68	58	59	58	60
	69	72	60	62	59	60
	71	74	65	66	61	63
	74	74	68	69	62	63
	75	77	73	74	67	68
	84	77	76	76	75	71
	85	83	79	76	77	72
	86	86	82	80	78	72
	87	88	83	83	80	77
	88	89	86	85	80	77
	91	91	87	89	84	80
	92	91	91	92	88	82
	93	97	93	94	89	83
	94	97	94	95	91	88
	99	101	95	97	94	91
	99	101	97	98	97	92
	101	104	98	98	98	92
	107	106	99	101	99	97
	108	107	100	104	101	100
	109	118	103	107	103	100
	117	121	103	109	106	106
	118	121	114	113	106	109
	123	122	122	116	111	113
	141	154	134	124	123	136
Mean of offspring.....	98.03 ± 0.73	93.9 ± 2.0	89.6 ± 1.9	84.8 ± 1.8		

corresponding decreases in the minus lines are only 5.7, 4.0 and 4.6.

Regression toward the mean of the unselected population decreases with successive selections. (Table 8.) The average regression in the three plus lines is 0.67 for the first selection,

TABLE 6  
*Minus selection*  
 Line F

GENERAL POPULATION	GENERATION 1		GENERATION 2		GENERATION 3	
	Males	Reduced females	Males	Reduced females	Males	Reduced females
Parents.....	89	78	76	71	61	64
Mid-parental values.....	83.5		73.5		62.5	
Offspring.....	63	51	48	59	58	47
	63	57	54	60	60	48
	70	63	59	63	61	53
	71	65	61	64	65	56
	73	68	67	69	67	60
	75	71	71	71	74	62
	75	72	83	75	78	66
	76	83	86	80	79	72
	78	85	89	80	80	74
	79	86	89	80	82	74
	84	88	90	82	88	79
	87	92	91	89	89	80
	88	94	91	91	89	83
	92	95	92	92	89	88
	96	100	95	95	90	89
	96	104	95	97	92	91
	98	107	97	98	94	91
	100	109	99	100	95	92
	102	115	102	103	98	94
	103	118	106	104	99	95
	104	118	107	104	101	100
	107	121	108	109	104	107
	110	125	110	112	110	116
	119	128	112	116	111	130
	159	151	143	122	114	137
Mean of offspring .....	98.03	$\pm$ 0.73	94.6	$\pm$ 2.2	89.6	$\pm$ 1.9
					84.7	$\pm$ 1.9

0.60 for the second and 0.57 for the third. For the minus lines the corresponding figures are 0.77, 0.64 and 0.60. On the other hand regression toward the mean of the parental generation increases with successive selections. (Table 9.) The average regression in this respect in the plus lines is 0.67 for the first

TABLE 7  
*Summary of tables 1 to 6*

	PARENTS		OFFSPRING					Number of individuals
	Mean number of facets	Mean number of facets	Standard deviation	Coefficient of variation	Extreme variates			
					high	low		
General population..	98.03	98.03±.73	24.3	24.8	182	45		500
Line A.								
Gen. 1.....	130.00	108.70±2.3	24.6	22.6	179	69		50
Gen. 2.....	174.00	127.50±3.1	32.9	25.8	210	84		50
Gen. 3.....	186.00	135.50±2.8	28.9	21.3	204	92		50
Line B								
Gen. 1.....	130.00	110.10±2.7	28.8	26.2	184	75		50
Gen. 2.....	174.50	128.60±3.2	33.4	26.0	222	79		50
Gen. 3.....	202.00	141.90±2.9	29.1	20.4	207	89		46
Line C								
Gen. 1.....	167.50	116.90±2.6	27.0	23.1	208	63		50
Gen. 2.....	183.50	133.50±3.3	34.3	25.7	198	67		50
Gen. 3.....	196.00	141.00±2.8	29.3	20.8	213	95		50
Line D								
Gen. 1.....	56.00	88.30±1.8	18.4	20.9	127	44		50
Gen. 2.....	70.00	85.50±1.8	18.9	22.1	133	49		50
Gen. 3.....	60.50	81.70±2.1	22.3	27.3	137	36		50
Line E								
Gen. 1.....	81.00	93.90±2.0	20.9	22.3	154	50		50
Gen. 2.....	69.00	89.60±1.9	20.1	22.5	134	50		50
Gen. 3.....	61.00	84.80±1.8	18.9	22.3	136	46		50
Line F								
Gen. 1.....	83.50	94.60±2.2	22.9	24.2	159	51		50
Gen. 2.....	73.50	89.80±1.9	20.3	22.6	143	48		50
Gen. 3.....	62.50	84.70±1.9	20.3	24.0	137	47		50

TABLE 8  
*Regression toward the mean of the unselected population*

	PLUS SELECTIONS				MINUS SELECTIONS				Average
	Line A	Line B	Line C	Average	Line D	Line E	Line F	Average	
Selection 1.....	0.67	0.62	0.73	0.67	0.77	0.76	0.77	0.77	
Selection 2.....	0.62	0.60	0.58	0.60	0.55	0.71	0.66	0.64	
Selection 3.....	0.57	0.58	0.57	0.57	0.56	0.63	0.62	0.60	

TABLE 9  
*Regression toward the mean of the parental generation*

	PLUS SELECTIONS				MINUS SELECTIONS			
	Line A	Line B	Line C	Average	Line D	Line E	Line F	Average
Selection 1 . . . . .	0.67	0.62	0.73	0.67	0.77	0.76	0.77	0.77
Selection 2 . . . . .	0.71	0.71	0.75	0.72	0.85	0.82	0.77	0.81
Selection 3 . . . . .	0.86	0.82	0.88	0.85	0.85	0.83	0.81	0.83

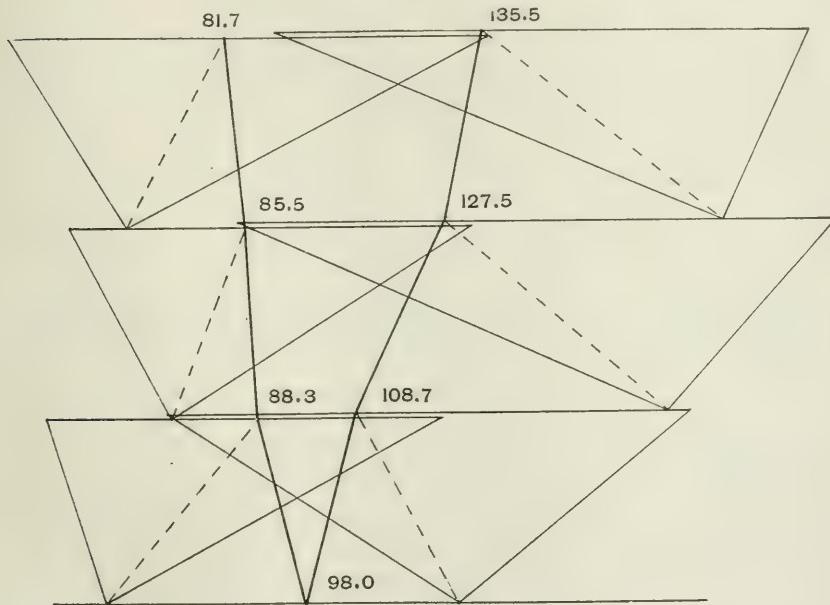


Fig. 3 The effect of selection in Plus Line A, at the right, and Minus Line D, at the left. The horizontal lines represent range of facet number, the lowest numbers at the left and the highest at the right. The lowest horizontal line represents the original unselected population, the others, beginning at the bottom, respectively the populations after the first, second and third selections. The extent of overlapping of facet number in each generation is indicated by the overlapping of the two parallel lines. The heavy lines give the progress of the mean values. The numbers are these mean values. A dotted line in every case runs from a mid-parental value, below, to the mean of the offspring, above. On the sides of each dotted line are the lines running from the mid-parental value to the values of the extreme individuals of the offspring. The full data are given in tables 1, 4 and 7.

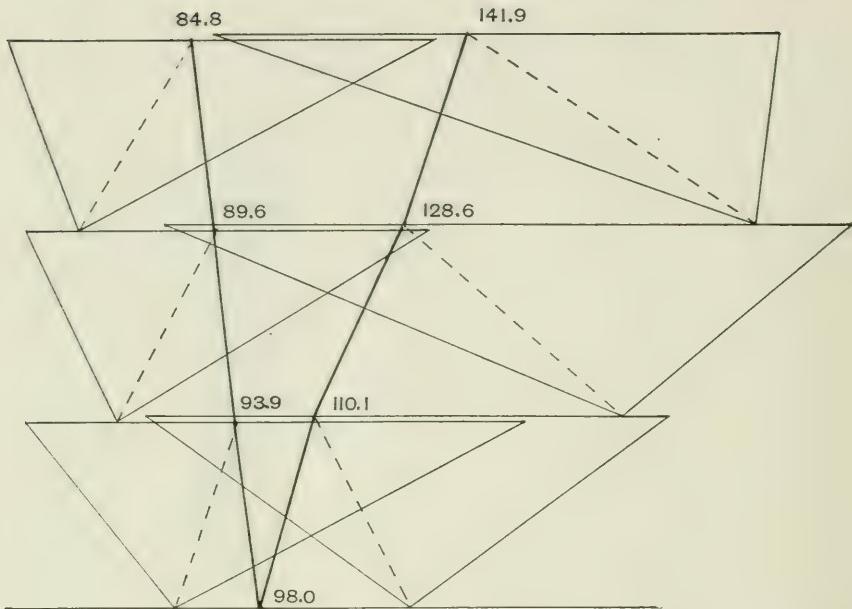


Fig. 4. The effect of selection in Plus Line B and in Minus Line E. See the description of figure 3 for further details. The full data are given in tables 2, 5 and 7.

selection, 0.72 for the second and 0.85 for the third. In the minus lines the corresponding figures are 0.77, 0.81 and 0.83. The decrease with respect to the mean of the general unselected population indicates that there is real progress during the successive selections. The increase with respect to the parental generation indicates that the effectiveness of the selection decreases with successive selections and that there is probably a limit to the number of effective selections. It seems probable that continued selection would not be able to change the number of facets in the mutant stock to that of the original stock from which it was derived.

The data presented thus show that selection in the 'bar eye' race of *Drosophila* is effective both in increasing and in decreasing the number of eye facets. As a result there can be no doubt of the existence of differences in the germinal constitution as

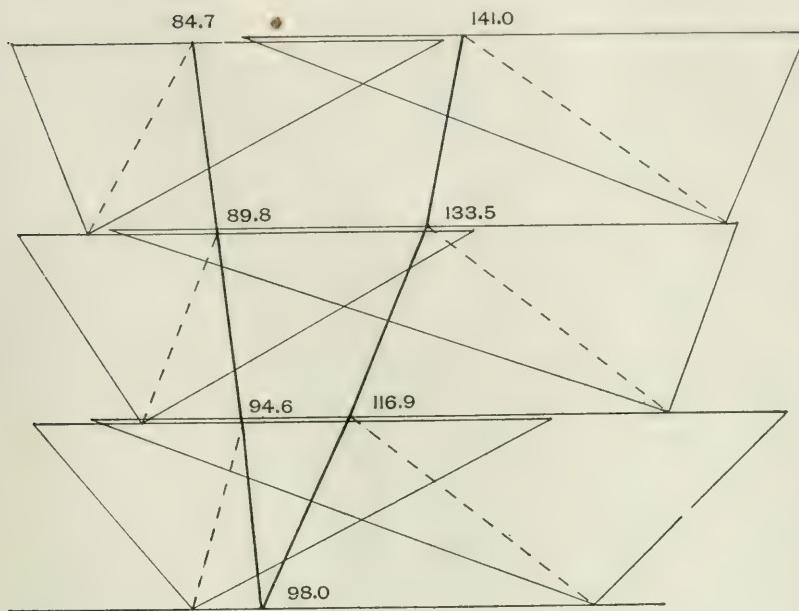


Fig. 5 The effect of selection in Plus Line C and in Minus Line F. See the description of figure 3 for further details. The full data are given in tables 3, 6 and 7.

regards this characteristic among the individuals of a generation. This fact is of special interest because the origin of the race by sudden appearance in a single individual is known and is of recent occurrence. Furthermore the behavior in crosses with the normal wild race shows that the mutant differs from the normal wild race in but a single Mendelian factor. If this were the only germinal factor involved in facet number we would be compelled to conclude that we have a case of variability in a unit factor. As far as the present selection data go such a provisional hypothesis would not be contrary to the facts. It seems more probable however that facet number in the normal wild race is represented in the germinal constitution by more than one factor and that the modification occurring in the production of the 'bar eye' race involved only one of these factors. That this factor is a most important one is of course indicated

by reduction from an average of 701.1\* facets in the original stock to 98.0 in the 'bar eye' stock. Selection, then, may have an effect because of variability or because of lack of homogeneity in the race as regards these other factors without regard to the 'barring' factor itself.

Three possibilities are thus open as regards the explanation of the effect of selection in this case. First, the 'barring' unit factor may be variable or may have varied since its first appearance in 1913. Second, the 'bar eye' race and by inference the original normal eyed stock from which it was derived may contain additional germinal factors affecting facet number and these additional factors may be variable. Third, the 'bar eye' race and by inference the original normal eyed stock from which it was derived may not be homogeneous with regard to these additional factors. Different factorial combinations may be present in different individuals. Selection in this case would segregate the 'high' combinations of factors on the one hand and the 'low' combinations on the other, yielding finally two homogeneous races in which further selection would have no effect. The 'highest' possible combination of factors as well as the 'lowest' possible may not exist in the original sample of the general population, but by Mendelian recombination it would finally appear.

While the data so far obtained do not enable us to decide which one of these three possibilities or which combination of them is to be considered as active in this case there is some evidence to support the view that the third is at least partly responsible. The increase in regression of the mean toward the mean of the parental population with each successive selection indicates an approaching limit to the effectiveness of selection. This is what we would expect in a population that is heterogeneous as regards factorial composition. If the unit factors themselves do not vary, selection must soon cease to have further effect.

## SUMMARY

1. Three successive selections for high number of facets in the 'bar eye' race of *Drosophila* increased the mean number of facets from 98.0 to 139.5.
2. Three similar selections for low number decreased the mean from 98.0 to 83.7.
3. The lowest individual, 89.0, in the 'high' lines after three selections is higher than the mean of the 'low' strains, 83.7, and the highest individual of the 'low' lines, 137.0, is lower than the mean of the 'high' strains, 139.5.
4. Significant progress was noted in each of the three selections in both 'high' and 'low' lines.
5. There are some differences in variability in the different generations but no significant change is proven.
6. Regression toward the mean of the general unselected population decreases with successive selections.
7. Regression toward the mean of the parental populations increases with successive selections. This increase makes it improbable that 'bar eye' stock can be raised to the original level by continued selection.
8. It is apparent from these data that individuals in any generation differ as regards germinal constitution.
9. If this difference in germinal constitution is solely in the unit factor concerned in 'barring,' variability in this unit factor must be assumed.
10. It is however more probable that there are other factors concerned in facet number. In that case the selection effect may be due either to variability of single unit factors or to presence of original differences in factorial composition. That it is due in part at least to the latter is indicated by the increase in regression toward the mean of the parental generation with successive selections.



# THE OCCURRENCE OF LETHAL FACTORS IN INBRED AND WILD STOCKS OF DROSOPHILA

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TWO DIAGRAMS

Three main points are dealt with in the following account:  
1) The relative frequency of sex linked lethal factors in inbred stocks of *Drosophila ampelophila* in comparison with their occurrence in wild stocks. 2) The occurrence of new lethals and their linkage relations to other sex linked characters. 3) The demonstration that an extraordinary sex ratio was due to the occurrence of two different lethal factors each carried by one of the sex chromosomes of the female that gave the ratio in question.

## THE RELATIVE FREQUENCY OF LETHALS IN INBRED AND WILD STOCKS

A hundred virgin females of a stock of *Drosophila* which was caught at Falmouth, Mass., in the summer of 1912, were mated individually on November 29, 1912, with males of the same stock. The counts of the offspring from each bottle are shown in table 1.

A hundred virgin females from a stock that had been caught at Falmouth, Mass., in the summer of 1911, were mated individually to males of the same stock early in January, 1913. The results are shown in table 3.

Numbers 13, 36, 38 and 47 of table 3 show a ratio of twice as many females as males while numbers 43, 53 and 67 are doubtful.

In order to determine whether the high ratios would reappear in later generations, virgin females from several of these cultures were mated to brothers. The results are shown in table 4.

Of the four sets of tests two (viz., 36 and 38) give ambiguous results, while numbers 13 and 47 give respectively 5 high to 9

TABLE 1

FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES
97	98	96*	59	85	81	105	105
134	147	121	113	93	93	88	79
77	79	94	99	73	77	58	47
121	117	116	98	105	108	71	75
138	144	82	82	34	41	92	82
132	129	183	166	112	104	47	45
99	111	117	107	72	74	56	62
122	142	161	160	85	92	93	80
127	128	147	140	52	47	89	77
64	61	62	51	87	92	73	87
122	152	126	120	88	79	96	92
95	110	105	112	68	77	69	70
75	82	50	37	133	112	62	74
103	120	109	111	72	93	154	142
112	120	94	97	126	112	203	172
135	137	112	94	117	123	175	147
42	47	138	141	102	102	165	154
166	150	75	74	98	101	120	86
129	127	100	110	91	80	49*	28
125	112	65	62	98	101	83	72
43	50	113	109	128	122	31	21
157	160	117	105	62	59	42	34
147	115	109	106	40	42	70	49
130	111	65	70	48	68	57	52
67	56	82	88	86	72	80	70

\* Two matings showed rather high sex ratios, and as this is often indicative of a lethal factor, I mated individually six of the virgin females from each bottle (twelve in all) to males from the same bottles. Since each bottle of the twelve (table 2) gave a 1 : 1 ratio it follows that the somewhat lower values noted in these two bottles was probably merely a chance deviation, or else due to the crowded condition of the bottle which prevented all of the males from hatching out. The males are known to continue hatching for some days after the females have ceased to hatch.

TABLE 2

26 F <sub>2</sub>		94 F <sub>2</sub>	
Females	Males	Females	Males
141	121	87	92
128	106	79	74
162	150	124	105
72	73	177	164
160	159	232	212
130	138	222	204

TABLE 3

NO. OF PAIR	FEMALES	MALES	SEX RATIO	NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	290	227	1.28 : 1	51	154	162	0.95 : 1
2	121	111	1.09 : 1	52	139	108	1.28 : 1
3	196	200	0.98 : 1	53	56	30	1.86 : 1
4	176	173	1.02 : 1	54	177	182	0.97 : 1
5	93	102	0.91 : 1	55	183	145	1.26 : 1
6	114	134	0.85 : 1	56	75	57	1.31 : 1
7	132	128	1.03 : 1	57	194	171	1.14 : 1
8	115	96	1.20 : 1	58	203	195	1.04 : 1
9	96	92	1.04 : 1	59	158	129	1.22 : 1
10	95	99	0.96 : 1	60	200	176	1.13 : 1
11	50	63	0.79 : 1	61	134	114	1.17 : 1
12	124	142	0.87 : 1	62	45	41	1.10 : 1
13	232	114	2.03 : 1	63	196	183	1.08 : 1
14	262	229	1.14 : 1	64	134	106	1.26 : 1
15	187	188	1.00 : 1	65	136	122	1.12 : 1
16	212	203	1.04 : 1	66	143	152	0.94 : 1
17	214	159	1.35 : 1	67	105	63	1.66 : 1
18	247	220	1.12 : 1	68	147	133	1.10 : 1
19	169	145	1.16 : 1	69	151	157	0.96 : 1
20	106	100	1.06 : 1	70	175	159	1.10 : 1
21	189	149	1.27 : 1	71	121	84	1.44 : 1
22	155	127	1.22 : 1	72	108	156	0.69 : 1
23	193	179	1.08 : 1	73	80	71	1.12 : 1
24	206	169	1.22 : 1	74	106	98	1.09 : 1
25	230	223	1.03 : 1	75	116	103	1.12 : 1
26	182	154	1.18 : 1	76	78	59	1.32 : 1
27	201	168	1.20 : 1	77	60	53	1.11 : 1
28	178	142	1.22 : 1	78	106	92	1.16 : 1
29	153	136	1.12 : 1	79	81	69	1.18 : 1
30	120	79	1.52 : 1	80	148	118	1.29 : 1
31	166	153	1.08 : 1	81	79	88	0.90 : 1
32	157	143	1.10 : 1	82	103	107	0.96 : 1
33	212	155	1.37 : 1	83	126	120	1.05 : 1
34	225	225	1.00 : 1	84	116	95	1.11 : 1
35	156	161	1.03 : 1	85	95	94	1.01 : 1
36	188	81	2.32 : 1	86	114	140	0.81 : 1
37	172	183	0.99 : 1	87	75	68	1.10 : 1
38	172	83	2.07 : 1	88	97	98	0.99 : 1
39	77	92	0.83 : 1	89	119	140	0.92 : 1
40	125	83	1.51 : 1	90	140	128	1.09 : 1
41	159	155	1.02 : 1	91	126	124	1.06 : 1
42	102	95	1.07 : 1	92	133	137	0.97 : 1
43	74	49	1.51 : 1	93	155	157	0.99 : 1
44	53	36	1.47 : 1	94	157	148	1.08 : 1
45	113	77	1.49 : 1	95	118	115	1.02 : 1
46	69	64	1.09 : 1	96	111	88	1.26 : 1
47	200	93	2.15 : 1	97	148	138	1.01 : 1
48	88	89	0.98 : 1	98	105	103	1.01 : 1
49	56	60	0.93 : 1	99	127	126	1.01 : 1
50	76	64	1.19 : 1	100	124	120	1.01 : 1

TABLE 4

13				36			
NO. OF PAIR	FEMALES	MALES	SEX RATIO	NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	194	95	2.04 : 1	1	116	128	0.91 : 1
2	123	50	2.46 : 1	2	114	124	0.92 : 1
3	123	124	0.99 : 1	3	89	63	1.41 : 1
4	131	113	1.16 : 1	4	101	107	0.94 : 1
5	164	61	2.70 : 1	5	100	74	1.35 : 1
6	108	86	1.14 : 1	6	102	124	0.82 : 1
7	143	53	2.70 : 1	7	119	96	1.24 : 1
8	82	104	0.79 : 1	8	92	94	0.98 : 1
9	193	173	1.11 : 1	9	70	49	1.43 : 1
10	148	82	1.80 : 1	10	100	80	1.25 : 1
11	159	143	1.11 : 1	11	194	153	1.27 : 1
12	87	93	0.94 : 1	12	109	47	2.32 : 1
13	53	59	0.96 : 1	13	129	105	1.23 : 1
14	119	173	1.50 : 1	14	124	105	1.18 : 1
15	165	152	1.10 : 1	15	127	116	1.10 : 1
38				47			
1	112	56	2.00 : 1	1	137	141	0.97 : 1
2	159	139	1.14 : 1	2	150	157	0.96 : 1
3	125	78	1.61 : 1	3	140	105	1.33 : 1
4	67	73	0.93 : 1	4	85	95	0.89 : 1
5	58	46	1.26 : 1	5	115	107	1.08 : 1
6	131	90	1.45 : 1	6	127	77	1.65 : 1
7	90	79	1.01 : 1	7	185	91	2.03 : 1
8	119	129	0.92 : 1	8	201	81	2.45 : 1
9	71	78	0.91 : 1	9	119	90	1.32 : 1
10	153	86	1.78 : 1	10	149	150	0.99 : 1
11	183	153	1.19 : 1	11	201	92	2.20 : 1
12	164	117	1.40 : 1				

low 1 doubtful and 4 high to 7 low ratios where equal numbers of each kind are expected. Further tests were therefore made. Pairs were mated from 13, 1; 13, 2; 13, 5; 13, 7; 36, 12; 47, 7; 47, 8; 47, 11.

The counts from these matings are shown in table 5.

As expected, the test of 13, 1 shows the presence of a lethal since there were 6 high to 6 low ratios.

The test of 13, 2 indicates a lethal with 8 high to 11 low ratios.

The test of 13, 5 indicates a lethal with 6 high to 13 low ratios.

TABLE 5

13, 1

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	186	103	1.80 : 1
2	118	122	0.97 : 1
3	121	128	1.06 : 1
4	116	101	1.15 : 1
5	171	81	2.11 : 1
6	138	46	3.00 : 1
7	120	116	1.03 : 1
8	107	52	2.06 : 1
9	115	110	1.05 : 1
10	104	128	0.81 : 1
11	127	68	1.86 : 1
12	174	100	1.74 : 1

13, 2

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	159	61	2.61 : 1
2	128	146	0.88 : 1
3	105	53	2.00 : 1
4	212	94	2.36 : 1
5	139	101	1.37 : 1
6	84	74	1.14 : 1
7	135	122	1.10 : 1
8	240	100	2.40 : 1
9	225	160	1.40 : 1
10	77	79	0.98 : 1
11	91	69	1.38 : 1
12	72	87	0.83 : 1
13	109	81	1.34 : 1
14	108	84	1.17 : 1
15	175	91	1.92 : 1
16	222	116	1.91 : 1
17	88	40	2.22 : 1
18	134	104	1.29 : 1
19	145	47	3.08 : 1

Two high to 9 low ratios appear in 13, 7, which probably indicates a lethal as the parent also had a high ratio (viz., 2.7 : 1).

These four tests of 13 show beyond doubt that a lethal was present, since each of the four families tested gave some high ratios.

TABLE 5—*Continued*

13, 5

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	116	139	0.87 : 1
2	103	100	1.03 : 1
3	222	109	2.03 : 1
4	140	140	1.00 : 1
5	159	58	2.75 : 1
6	150	168	0.89 : 1
7	205	210	0.98 : 1
8	107	44	2.43 : 1
9	239	140	1.71 : 1
10	164	137	1.20 : 1
11	125	117	1.07 : 1
12	148	123	1.20 : 1
13	172	156	1.10 : 1
14	107	50	2.14 : 1
15	113	115	0.98 : 1
16	100	134	0.75 : 1
17	211	150	1.41 : 1
18	116	133	0.87 : 1
19	155	62	2.50 : 1

13, 7

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	267	229	1.17 : 1
2	180	161	1.11 : 1
3	136	123	1.10 : 1
4	208	89	2.32 : 1
5	106	106	1.00 : 1
6	162	87	2.00 : 1
7	139	131	1.06 : 1
8	119	140	0.85 : 1
9	121	119	1.01 : 1
10	109	76	1.43 : 1
11	113	83	1.36 : 1

Of the 60 females tested, half (30) should have given 2 : 1 ratios, while in fact, only 22 gave such ratios to 38 giving normal ratios. Provisionally, this deviation may be ascribed to chance.

The following tests were made of the daughters of number 12 of 36, table 4:

TABLE 5—*Continued*

36, 12

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	121	72	1.7 : 1
2	151	56	2.8 : 1
3	130	109	1.2 : 1
4	159	140	1.1 : 1
5	97	115	0.8 : 1
6	177	108	1.6 : 1
7	135	88	1.5 : 1
8	144	55	2.6 : 1

In this test there were 5 high to 3 low ratios showing that 36, 12 was a lethal bearing female.

The following tests were made of the daughters of number 7 of 47, table 4.

TABLE 5—*Continued*

47, 7

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	259	121	2.1 : 1
2	235	122	1.9 : 1
3	188	78	2.4 : 1
4	133	152	0.9 : 1
5	170	90	1.9 : 1
6	185	95	1.9 : 1
7	147	148	1.0 : 1
8	164	150	1.1 : 1

Here there are 5 high to 3 low ratios indicating a lethal.

In 47;8 the 4 high to 4 low ratios indicate a lethal.

In 47, 1 there are 3 high to 7 low ratios. Of the three sets of tests of 47 of table 5 there were 12 high to 14 low ratios which establishes the presence of a lethal in this line. The general outcome of these tests leaves no doubt that a lethal was present in the original females that were tested.

Since only half of the daughters of a lethal female are heterozygous for lethal and since these females are indistinguishable

TABLE 5—*Continued*

47, 8

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	146	80	1.8 : 1
2	93	94	1.0 : 1
3	201	162	1.2 : 1
4	124	58	2.1 : 1
5	147	156	0.9 : 1
6	196	69	2.8 : 1
7	150	119	1.3 : 1
8	158	57	2.8 : 1

47, 11

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	142	79	1.79 : 1
2	168	173	0.98 : 1
3	157	171	0.92 : 1
4	159	162	0.98 : 1
5	167	124	1.34 : 1
6	247	124	2.00 : 1
7	142	127	1.12 : 1
8	190	97	1.96 : 1
9	122	146	0.90 : 1
10	163	156	1.04 : 1

from their sisters, it is by chance only that one would choose a female with a lethal factor when testing out a stock. On the other hand if a lethal female is mated to a male having a sex

TABLE 6

13, 1, 11

NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	183	156	1.11 : 1
2	138	118	1.17 : 1
3	155	70	2.22 : 1
4	158	139	1.12 : 1
5	154	82	1.90 : 1
6	142	152	0.90 : 1
7	163	152	1.01 : 1

TABLE 6—Continued

13, 1, 12

1	114	70	1.63 : 1
2	156	136	1.12 : 1
3	199	108	1.83 : 1
4	154	82	1.88 : 1
5	195	102	1.92 : 1
6	154	103	1.50 : 1

13, 2, 1

1	123	60	2.00 : 1
2	130	52	2.50 : 1
3	98	105	0.93 : 1
4	82	3	27.33 : 1
5	110	95	1.15 : 1
6	140	135	1.04 : 1
7	170	50	3.40 : 1
8	84	62	1.35 : 1
9	99	70	1.41 : 1

13, 2, 8

1	83	61	1.36 : 1
2	150	62	2.42 : 1
3	105	96	1.08 : 1
4	156	162	0.97 : 1
5	84	39	2.16 : 1
6	134	134	1.00 : 1
7	120	73	1.64 : 1
8	109	64	1.54 : 1
9	134	111	1.21 : 1

13, 2, 19

1	164	129	1.27 : 1
2	207	214	0.92 : 1
3	225	211	1.07 : 1
4	232	172	1.35 : 1
5	182	185	1.00 : 1
6	341	120	2.84 : 1
7	283	152	1.86 : 1
8	193	93	2.07 : 1
9	366	167	2.20 : 1
10	251	246	1.02 : 1

linked factor close to the lethal factor in question a stock may be obtained in which the lethal females may be selected with great probability. For example: If a red eyed female carrying a lethal is mated to a white eyed male half of her daughters will have the factor for red and the factor for lethal in one X chromosome and the factor for white and the factor that is the normal allelomorph of lethal in the other chromosome. If such a daugh-

TABLE 7  
13, 2, 8, 2 (*four 1 : 1 ratios omitted*)

NO. OF PAIR	FEMALES		MALES		CROSSING OVER BETWEEN WHITE AND LETHALS
	Red	White	Red	White	
1	62	73	11	47	19.0
2	87	78	12	48	20.0
3	99	77	23	58	28.4
4	77	67	11	55	16.7

13, 2, 19, 6 (*five 1 : 1 ratios omitted*)

1	177	94	30	88	25.4
2	110	117	19	81	19.0

13, 2, 19, 7 (*three 1 : 1 ratios omitted*)

1	83	107	23	69	25.0
2	112	118	22	93	19.1
3	98	81	26	68	27.7
4	162	85	14	79	15.1
5	89	94	20	71	22.0
6	98	87	27	72	27.3
7	82	83	18	65	21.7

ter is mated to a white eyed male half of the female offspring will be red eyed and half white eyed. The former getting their red bearing chromosome from their mother will be the lethal bearing females since the red bearing chromosome also carried the lethal factor. By selecting the red eyed females, therefore, in each succeeding generation and breeding them to white eyed males the lethal stock can be maintained.

Virgin females from numbers 13, 1, 11; 13, 1, 12; 13, 2, 1; 13, 2, 8; 13, 2, 19; of table 5 were mated to white eyed males. The results are shown in table 6.

The tests give 19 high to 22 low ratios which is the expectation for lethals, i.e., equality is expected and is approximately realized.

Daughters from 2 : 1 cultures all of which were heterozygous for white and half of which should be heterozygous for lethal also, were again mated to white eyed males with the results in table 7.

There were 13 high to 13 low ratios shown by these daughters indicating a lethal factor. On the basis of these data the locus of the lethal is at 23.7.

It is interesting to note that the lethal factor occurred in flies that had been inbred a year and that none appeared in the stock having been inbred only two months. Miss Rawls (*Biol. Bull.* '13) found her lethal in a stock that had been inbred a year. The lethals described by Quackenbush (*Sc.* '10) and Morgan (*Sc.* '12; and *Jour. Exp. Zoöl.* '14) appeared in stocks that had been inbred for some time. To test whether, in general, lethals are more frequent in inbred stocks I mated 100 pairs from wild stock caught at Falmouth, Mass., and 70 pairs from wild stock caught at Harris, Minn. The results are shown in table 8.

Tables 1 and 8 show that the counts made of offspring from 270 pairs of fresh wild stocks have no unusual ratios.

#### THE SECOND LETHAL FACTOR

On February 10 of 1914, sixty pairs from stock collected in the summer of 1910 were mated. The results are shown in table 9.

The next to the last pair of the above table seemed to show the presence of a lethal factor. Sixty virgin daughters from this pair were mated to brothers. Nearly one-half of these gave a ratio of twice as many females as males, as shown in table 10.

Virgin females from numbers 7, 8, 12, 23 and 59 were mated to white eyed males. About one-third of the counts of the next generation show the presence of a lethal factor (table 11). Some of these lethal females were again mated to white eyed

TABLE 8 (a)  
*Falmouth Stock*

FEMALES	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES
98	111	129	111	161	132	175	182
144	132	207	216	175	176	171	174
74	78	181	154	195	187	191	190
68	86	182	193	101	118	199	223
129	120	196	205	102	85	100	88
120	117	179	187	154	162	99	106
119	138	191	175	195	190	185	180
178	174	105	97	191	198	100	84
176	175	184	181	199	202	174	194
96	103	173	138	150	150	87	128
130	109	186	150	166	183	91	90
156	141	174	169	110	123	98	86
103	88	170	197	175	177	100	108
125	152	187	222	176	180	100	93
105	95	220	182	192	171	146	136
84	92	186	157	189	172	108	110
140	188	95	110	175	193	100	92
128	151	192	200	159	173	85	102
127	139	174	171	171	159	100	101
157	193	113	111	177	173	172	160
120	145	167	119	166	184	183	189
109	106	123	120	117	81	192	189
102	100	191	210	159	163	176	180
148	125	134	129	110	107	164	170
167	155	106	102	191	199	171	175

males. It was expected that only a few of the red eyed males would appear in the second generation if red and lethal were closely linked. Results (table 11) show, however, that a great number of red males appear. Such a result indicates that a new lethal had appeared which was located some distance from the factor for white, thus giving a chance for a greater amount of crossing over.

In table 11 it will be noted that the percentage of crossovers is 46. This would place the lethal somewhere beyond the factor for sable and not far from the factor for bar eyes upon the sex chromosome.

TABLE 8 (b)

*Harris Stock*

FEMALES	MALES	FEMALES	MALES	FEMALES	MALES
69	166	137	142	83	95
126	126	117	96	176	167
158	139	104	104	91	86
124	121	97	105	175	130
159	216	115	125	140	139
163	132	105	98	94	76
180	170	115	79	138	120
199	196	84	87	116	115
154	155	93	81	183	185
153	153	115	89	163	135
157	167	93	75	107	78
165	135	104	105	138	120
104	117	93	82	164	161
183	179	110	136	218	229
136	142	112	110	215	210
170	156	139	129	211	196
173	161	144	132	240	244
151	133	90	87	195	236
98	98	125	93	162	165
129	99	88	82	102	98
122	116	121	119	236	230
106	95	137	117	144	154
70	90	134	135	166	167
96	79	122	96	172	165

To determine the approximate location of the lethal upon the chromosome virgin females of the fifth generation (table 11) were mated, some to sable males, and some to bar eyed males.

The counts of the second generation of crosses with sable and with bar eyed males are shown in table 12. The 1 : 1 ratios are omitted.

The data in this table show that the distance of this lethal from bar is 8.3.

If the locus of the factor for sable is 43 and that for bar is 57, the data of table 12 show that the locus of the new lethal is  $43 + 23.5$  or 66.5, since the distance of lethal from the factor for bar is only 8.3. The locus of the lethal must be 'to the right' of both factors since  $43 + 23.5$  or 66.5 is approximately equal

TABLE 9

FEMALES	MALES	FEMALES	MALES	FEMALES	MALES
146	151	164	161	164	169
157	168	82	125	148	131
83	79	183	136	266	239
88	99	197	193	201	216
95	104	114	93	167	160
108	103	114	111	133	129
76	78	96	106	112	140
140	138	88	86	106	86
190	213	93	120	116	97
105	85	114	95	169	173
100	79	155	230	199	185
176	152	106	111	181	205
131	155	98	103	145	154
102	84	142	119	106	96
160	133	91	64	110	96
171	153	138	110	181	197
250	188	143	135	195	178
252	216	152	135	142	162
150	163	180	149	266	108
139	105	148	134	86	55

to  $57 + 8.3$  or 65.3 or at 65.6. The locus indicated by both experiments when the data are weighted proportionately and a correction is made for double crossing over is 66.2.

In the spring of 1914, an interesting lethal turned up in my 1910 stock. Half of the males hatched out as normal males but of the other half, though able to pass through the different stages of metamorphosis, many of them were unable to escape from the pupa case. Those that chanced to do so fell over on one side when trying to walk. I examined all the appendages carefully but noticed no abnormalities. Nevertheless, the legs did not seem strong enough to support the body, nor did they seem to move coördinately and for that reason would so often become entangled with one another that the fly could not get them separated and would die from exhaustion in a day or so. Not any of these males lived longer than two days.

Whether the first lethal of the 1910 and the lethals of the 1911 flies allow any development of the lethal bearing male at all, is still under investigation.

TABLE 10

NO. OF PAIR	FEMALES	MALES	SEX RATIO	NO. OF PAIR	FEMALES	MALES	SEX RATIO
1	211	99	2.13:1	31	112	49	2.28 : 1
2	197	87	2.26:1	32	56	54	1.03 : 1
3	161	91	1.77:1	33	176	103	1.70 : 1
4	123	83	1.48:1	34	46	51	0.90 : 1
5	201	100	2.00:1	35	143	78	1.86 : 1
6	64	76	0.84:1	36	44	43	1.00 : 1
7	277	129	2.15:1	37	205	128	1.60 : 1
8	253	125	2.02:1	38	49	50	1.00 : 1
9	63	44	1.43:1	39	66	27	2.19 : 1
10	112	67	1.67:1	40	26	23	1.13 : 1
11	96	48	2.00:1	41	73	66	1.10 : 1
12	244	116	2.10:1	42	28	14	2.00 : 1
13	155	92	1.68:1	43	160	36	4.44 : 1
14	69	67	1.03:1	44	124	51	2.43 : 1
15	72	73	1.00:1	45	109	43	2.30 : 1
16	119	69	1.72:1	46	45	42	1.00 : 1
17	168	102	1.64:1	47	50	44	1.14 : 1
18	71	50	1.42:1	48	173	90	1.92 : 1
19	38	12	3.00:1	49	115	78	1.47 : 1
20	90	36	2.50:1	50	44	28	1.58 : 1
21	213	141	1.51:1	51	64	30	2.00 : 1
22	70	60	1.16:1	52	58	64	0.90 : 1
23	208	90	2.31:1	53	164	74	2.21 : 1
24	117	74	1.58:1	54	73	70	1.00 : 1
25	64	61	1.05:1	55	27	29	0.93 : 1
26	85	60	1.41:1	56	103	72	1.34 : 1
27	69	35	1.96:1	57	56	62	0.90 : 1
28	88	69	1.28:1	58	191	118	1.61 : 1
29	231	123	1.88:1	59	193	88	2.19 : 1
30	217	119	1.82:1	60	148	85	1.74 : 1

Since no lethal has been found in the hundreds of pairs of fresh wild stock examined and since lethals have occurred in many of the inbred stocks, it may appear that inbreeding (with its constraints of confinement and homogeneous feeding) tends to cause the mutation to appear or else the removal of the flies from the competition that takes place under wild conditions makes possible the preservation and continuance of any lethal factors that may appear.

A discussion of these alternatives would involve a fuller knowledge than we have at present of the causes of mutations and the

TABLE 11

1910 Lethal	No. of Pair			No. of Pair		
		♀s	♂s		♀s	♂s
7 (277	129)	$\times W \rightarrow$		1 (208	104)	
				4 (178	76)	
				7 (169	69)	$\times W \rightarrow$
8 (253	125)	$\times W \rightarrow$		6 (229	107)	
				10 ( 52	19)	$\times W \rightarrow$
				12 (258	127)	
				13 (207	107)	
F <sub>1</sub>				7 (146	93)	$\times W \rightarrow$
♀s				4 (189	99)	$\times W \rightarrow$
(266	108)			8 (170	94)	$\times W \rightarrow$
23 (208	90)	$\times W \rightarrow$		6 (147	93)	
				8 (228	92)	$\times W \rightarrow$
59 (193	88)	$\times W \rightarrow$		1 (228	101)	$\times W \rightarrow$
				10 (210	97)	$\times W \rightarrow$
				13 (212	105)	$\times W \rightarrow$

\* The 1 : 1 ratios are omitted.

TABLE 11

No. of Pair	R♀s	R♂s	W♀s	W♂s		No. of Pair	R♀s	R♂s	W♀s	W♂s
1 (148)	75	166	123)		$\times W$	1 (148)	56	135	77)	
4 (220)	92	124	135)			3 (128)	29	107	61)	
6 (179)	69	144	96)			4 (131)	46	132	85)	
7 (160)	63	150	80)			6 (132)	42	92	68)	
						7 (159)	54	121	101)	
						8 (158)	61	152	79)	
2 (170)	80	74	90)		$\times W$	1 ( 67	27	55	37)	
5 (140)	44	146	93)			4 ( 88	26	78	51)	
						5 (142)	54	96	64)	
						6 (105)	45	92	43)	
						8 ( 65	20	76	35)	
						9 (119)	61	93	61)	
6 (175)	64	201	95)							
7 (120)	44	122	66)							
9 (109)	66	118	46)							
1 (133)	56	135	68)		$\times W$	3 (151	52	135	82)	
3 (183)	76	145	97)			5 (134	59	117	85)	
4 (161)	64	162	89)			6 (132	47	117	73)	
7 (160)	57	165	84)			7 (130	53	126	67)	
						8 (124	57	112	55)	
						9 (128	61	137	68)	
						10 (101	42	80	56)	
1 (138)	68	146	77)							
1 (113)	61	118	58)							
2 (166)	80	170	91)							
5 (116)	57	212	146)							
10 (147)	57	108	83)		$\times W$	5 (114	47	96	62)	
11 (153)	67	139	79)			6 (108	56	103	63)	
12 (224)	83	194	86)			9 (135	53	124	74)	
15 (120)	60	124	69)			11 (124	52	103	56)	
						12 (139	60	141	72)	
2 (121)	45	106	72)			2 (109	34	139	81)	
4 (113)	64	115	63)							
6 (124)	95	124	62)			3 ( 84	38	80	48)	
9 (117)	49	131	57)		$\times W$	6 (108	46	90	57)	
10 (173)	62	96	56)			7 (125	67	112	63)	
11 (130)	56	109	59)			12 (135	49	78	70)	
13 (121)	54	123	69)			13 (109	42	77	58)	
4 (113)	56	111	71)		$\times W$	7 (76	17	51	33)	
6 (139)	50	134	76)				1405		1648	
8 (117)	42	112	63)							
9 (124)	65	113	65)							

Total no. of ♂'s = 3053      No. of cross overs = 1405  
The ratio of the cross overs to all the ♂'s = 1405 ÷ 3053 = .46

frequency of their appearance. For the present, therefore, the fact of the occurrence of the lethals in the inbred stocks is the one important result of this examination.

#### THE PRESENCE OF TWO LETHALS

Number 4 of 13, 2, of table 6 yielded a ratio of 27.33 : 1. It seemed probable in this case that two different lethal factors were present, one in each chromosome. This might seem to prevent all the sons from developing since each son must get one or the other maternal sex chromosome; but the survival of the three males would be possible through the crossing over

TABLE 12 (a)

Heterozygous Bar Female  $\times$  Bar Male

NO. OF PAIR	FEMALES		MALES		Crossover Percentage
	Bar		Bar	Normal	
1	284		108	5	4.4
2	214		93	13	12.3
3	120		47	3	6.0
4	187		86	12	12.2
5	173		72	4	5.3
6	146		64	8	11.1
7	187		78	8	9.4
8	247		122	5	4.2
9	138		91	4	4.2
10	166		52	4	7.1
11	167		74	11	13.0
12	183		75	12	13.0
13	123		75	10	12.0
14	230		129	10	7.1
15	121		56	3	5.0
16	193		60	7	10.0
17	241		107	10	8.0
18	159		84	2	2.3
19	143		77	9	10.0
20	139		50	4	8.0
		1590		144	

Total number of ♂'s, 1734; Crossovers = 144.

Crossover percentage =  $144 \div 1734$  or 8.3.

TABLE 12 (b)  
Red eyed ♀ heterozygous for sable and lethal × sable ♂

NO. OF PAIR	SABLE	NORMAL	SABLE	NORMAL	CROSSOVER PERCENTAGE
1	45	40	35	6	15.0
2	111	101	80	20	20.0
3	167	112	84	30	26.3
4	110	74	69	21	24.6
5	162	104	95	37	28.0
6	77	59	45	9	17.0
7	149	113	92	22	20.0
8	92	104	58	25	29.0
9	91	105	65	25	27.7
10	85	78	55	16	22.5
11	134	115	78	29	27.7
12	111	113	75	21	22.1
13	138	138	89	33	27.0
14	35	38	35	5	12.5
15	27	52	29	8	21.6
16	121	114	63	21	25.3
17	54	71	48	14	22.6
18	86	91	80	26	24.5
19	73	83	79	19	19.4
			1254	387	

The total number of males = 1641; Crossovers = 387.

The crossover percentage =  $387 \div 1641$  or 23.5.

Therefore, the distance of lethal from sable is 23.5.

of one of the lethal factors from one chromosome in the other, thus freeing one chromosome from its lethal factor.

TABLE 13  
13, 2, 1, 4

NO. OF PAIR	FEMALES		MALES		CROSSING OVER BETWEEN WHITE AND LETHAL	SEX RATIO
	Red	White	Red	White		
1	172	174	32	116	21.6	2.34
2	217	228	30	136	18.1	2.72
3	147	174	29	126	18.7	2.07
4	215	216	28	174	13.9	2.13
5	229	194	44	124	26.2	2.52
6	228	209	30	144	17.2	2.51
7	184	201	35	139	20.1	2.21
8	214	212	25	147	14.5	2.47
9	193	155	38	138	21.6	1.97

Nine of the daughters were mated individually to white eyed males and gave the results in table 13.

All nine daughters gave a 2 : 1 ratio which is expected if their mother had two lethals.

Ten other daughters (from the very high ratio mother) were mated to their red eyed brothers<sup>1</sup> and gave the results in table 14.

Six red females of table 13 were tested and gave the following results:

13, 2, 1, 4, 7

NO. OF PAIR	FEMALES		MALES		CROSSING OVER BETWEEN WHITE AND LETHAL	SEX RATIO
	Red	White	Red	White		
1	59	51	4	47	7.8	2.3 : 1
2	88	84	8	59	12.0	2.6 : 1
3	87	66	12	72	15.4	1.8 : 1

13, 2, 14, 8

1	90	80	11	60	15.5	2.3 : 1
2	73	79	10	73	12.0	1.8 : 1
3	75	66	11	63	15.1	1.9 : 1

Three of the sisters by brother No. 1 failed to produce any progeny when transferred to separate bottles. Each of the other sisters, however, showed the presence of a lethal factor. Thus all 19 daughters of the female gave a 2 : 1 ratio. There can be no question but that the high sex ratio of the mother was due to two lethals. Virgin red eyed daughters of some of these females were then mated to white eyed brothers with results as shown in table 15.

Since the mothers of the females used in table 15 were all heterozygous for white and for one or the other lethal, they would, when mated to red eyed males, produce two kinds of daughters: one-half heterozygous for a lethal and the other half

<sup>1</sup> If the sons that came through were due to crossing over, then the X chromosome that went into each is free from lethals and consequently they must be normal males. The normality of these three males was also tested by mating them to wild females. The sex ratio was normal. The daughters of the three males were tested individually and gave normal ratios.

TABLE 14  
*Ten sisters by brother No. 1*

NO. OF PAIR	RED FEMALES	RED MALES	WHITE MALES	SEX RATIO	CROSSING OVER BETWEEN WHITE AND LETHAL
1	105	8	41	2.14 : 1	16.7
3	224	23	108	1.80 : 1	17.6
5	64	6	18	2.70 : 1	25.0
6	90	7	36	2.09 : 1	16.3
7	35	2	7	3.88 : 1	22.2
8	38	2	6	4.75 : 1	25.0
9	36	3	12	2.40 : 1	20.0
Mass.....	428	61	136	2.18 : 1	30.9

*Three sisters by brother No. 2*

1	128	18	38	2.0 : 1	32.1
2	176	12	78	2.0 : 1	13.3
3	197	11	78	2.1 : 1	12.4
Mass.....	336	25	109	2.5 : 1	18.6

heterozygous for white. The females heterozygous for white when mated to white eyed males would produce equal numbers of red eyed and white eyed males and females (table 15) except where the lethal may have crossed over to the factor for white (as was the case in numbers 5 and 1, starred in table 15).<sup>2</sup> The females heterozygous for lethal when mated to the white eyed males produce the 2 : 1 ratio, also indicated in table 15.

Matings were, also, made between the three males and daughters of the sisters of the males with results as follows:

(13, 2, 1, 4; Female by W male)F; Female by No. 2 male

NO. OF PAIR	FEMALES	MALES		SEX RATIO	CROSSING OVER BETWEEN WHITE AND LETHAL
		Red	White		
Mass	236	6	95	2.3 : 1	5.9
1	172	16	85	1.7 : 1	15.8
2	116	9	43	2.2 : 1	17.3
3	125	7	40	2.7 : 1	14.9
4	141	5	61	2.1 : 1	7.6

<sup>2</sup> The other class resulting from such crossing over should contain neither the white nor the lethal, and one such female is recorded in table 15.

TABLE 15  
(*Sister by No. 1 male*), F<sub>2</sub>

NO. OF PAIR	FEMALES		MALES		SEX RATIO	CROSSING OVER BETWEEN WHITE AND LETHAL
	Red	White	Red	White		
1	72	80	69	78	1.02 : 1	
2	63	48	49	49	1.13 : 1	
3	18		8		2.25 : 1	
4	50		32		1.56 : 1	
5*	43	51	27	6	2.85 : 1	18.2

(*Sister by No. 1 male*) 3 F<sub>2</sub>

1	145		72		2.00 : 1	
2	141		75		2.00 : 1	
3	53	67	47	75	1.00 : 1	
4	30	42	32	41	1.00 : 1	
5	43	34	50	44	0.82 : 1	
6	161	34	67	44	1.76 : 1	

(*Sister by No. 2 male*), F<sub>2</sub>

1	137		72		1.90 : 1	
2	103		107		0.90 : 1	
3	72	86	81	74	1.00 : 1	
4	164		86		2.00 : 1	
5	101		47		2.10 : 1	

(*Sister by No. 3 male*), 3 F<sub>2</sub>

1*	101	85	64	14	2.40 : 1	19.0
2	29	24	24	20	1.00 : 1	
3	168		65		2.60 : 1	

(*Sister by No. 2 male*) Mass F<sub>2</sub>

1	97	69	77	70	1.00 : 1	
2	78		24		3.00 : 1	
3	153		72		2.00 : 1	

The four daughters show the presence of a lethal.

Summary: The nineteen tested females of 13, 2, 1, 4 (table 6) gave a 2 : 1 ratio. Other tests showed that the three males behaved like normal males. The explanation of her high ratio (2 : 1) is that two lethal factors were present.

Table 16 gives some of the counts of the descendants of 13, 2, 1, 4 with white eyed males.

TABLE 16

FEMALES		MALES		CROSSING OVER BETWEEN WHITE AND LETHAL
Red	White	Red	White	
162	85	14	79	15.0
228	209	30	144	17.2
100	84	13	68	16.0
91	91	12	68	15.0
127	123	15	85	15.0
122	125	16	108	12.8
127	110	18	117	13.3
115	115	23	119	16.2
95	86	12	57	17.4
142	105	15	88	14.5
137	138	20	118	14.4
43	43	8	39	17.0
87	66	12	72	14.3
90	70	11	60	15.5
108	102	17	79	17.7
72	65	15	53	22.0
76	77	13	48	21.3
120	140	30	120	20.0
119	100	29	90	24.3
130	98	18	69	20.7
149	133	22	95	18.8
193	155	38	138	21.6
229	194	44	124	26.2
172	174	32	116	21.6
193	155	38	138	21.6
184	201	35	139	20.1

Numbers 5 and 1 starred of table 15 show a decided decrease in the number of white males. It looked as if the lethal factor had crossed over into the chromosome carrying the factor for white. To examine this, virgin females from number 1 were mated to white eyed miniature males as shown in table 17.

A lethal factor connected with the factor for white is evidently present since all the white eyed females gave a 2 : 1 ratio.

The red eyed sisters of the white eyed females in table 17a should bear no lethal if, as the last table indicates, crossing over

TABLE 17 (a)

*White eyed granddaughters of sister and brother by a white eyed miniature male*

NO. OF PAIR	WHITE FEMALES	WHITE MALES	SEX RATIO
1	79	44	1.8 : 1
2	171	71	2.4 : 1
3	176	88	2.0 : 1
4	182	90	2.0 : 1
5	187	88	2.1 : 1
6	176	63	2.8 : 1
7	139	48	2.9 : 1
8	182	72	2.5 : 1
9	170	104	1.6 : 1
10	164	68	2.4 : 1

had taken place, except when crossing over occurred again in the mother. This was tested (table 17b).

TABLE 17 (b)

*Red eyed granddaughters of sister and brother by white eyed miniature male*

NO. OF PAIR	FEMALES		MALES		SEX RATIO
	Red	White	Red	White	
1	48	59	20	43	1.54 : 1
2	76	78	74	58	1.16 : 1
3	82	59	50	50	1.41 : 1
4	69	68	66	62	1.07 : 1
5	76	52	67	51	1.08 : 1
6	65	63	80	61	0.90 : 1
7	88	53	29	59	1.60 : 1
8	33	34	5	27	2.09 : 1
9	42	53	6	35	2.32 : 1
10	79	65	1	44	3.20 : 1
11	64	99	7	71	2.09 : 1

Four of the red eyed females gave a 2 : 1 ratio indicating that crossing over did occur in the mother.

To determine whether this lethal is the new one or the original one whose locus was shown to be at 23.7 the white eyed females were mated to eosin<sup>3</sup> to find the locus of the factor in the chromo-

<sup>3</sup> Eosin is an allelomorph of white. A female that has the eosin factor in one X and the white factor in the other X can be distinguished from a pure female with eosin in both X's.

somes. If it is a crossover it should be found to have the same locus as the factor with the red.

The white eyed females mated to eosin males gave white-eosin<sup>3</sup> long winged females (with the factor for lethal); white-eosin miniature females; white eyed miniature males; and a few white eyed long winged males as crossovers.

The white-eosin long winged females were again mated to eosin miniature males. This mating gave white-eosin long winged females with the lethal factor, eosin miniature females without the lethal factor, eosin long winged females and, white-eosin miniature females, eosin miniature males, white eyed miniature males, eosin long winged males and white long winged males. The single crossovers are the white miniature and the eosin long winged males, while the white long winged males are double crossovers. In table 18 some of the counts are given.

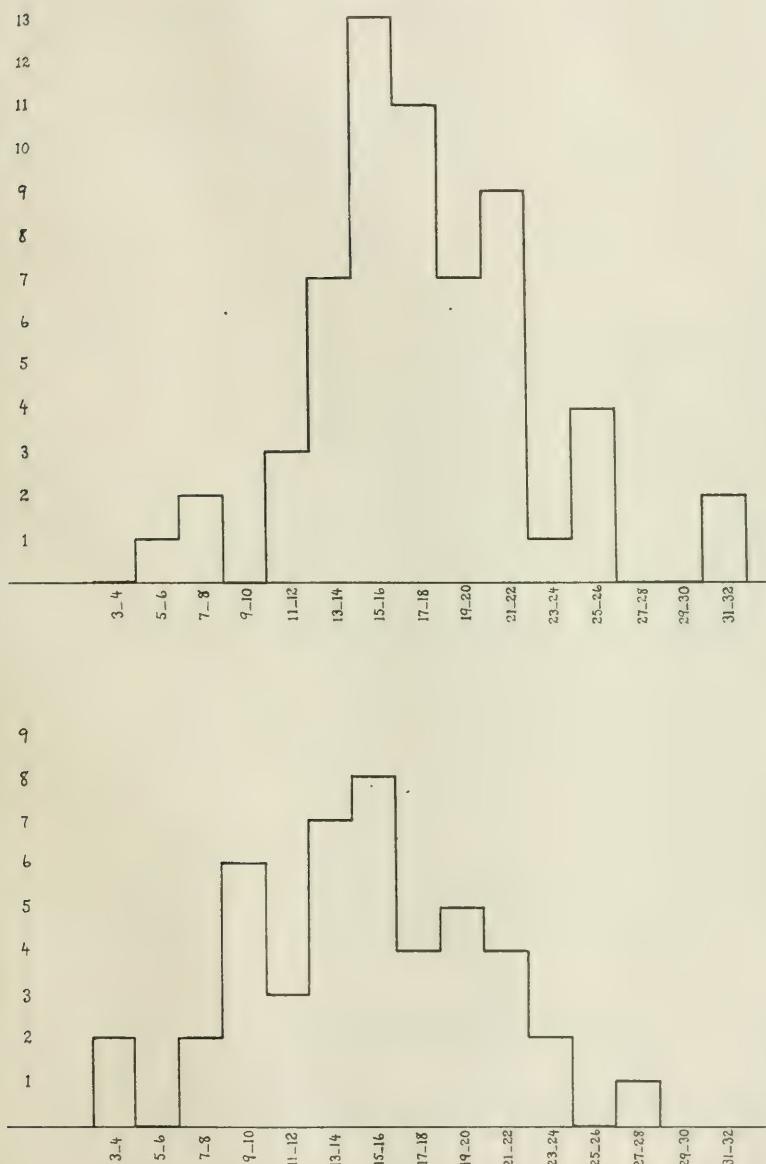
The crossover value of white with the lethal involved is 15.6 and that of the lethal with miniature is 19.9. Therefore the locus of this lethal is at 16.7, that is, 1.1 plus 15.6. The locus of the original lethal was shown to be at 23.7, so that this lethal with a locus at 16.7 must be the new lethal whose advent led to the production of the high sex ratio. The mother of the high sex ratio carried in one of her X chromosomes the original lethal at 23.7 and in the other X chromosome, the one derived from the father, the new lethal at 16.7.

If this female contained two lethals some of her descendants should have one and some the other; and these two kinds should be expected to give slightly different linkage values with whit<sup>2</sup>. If, then, the values obtained from all of her descendants be plotted they should give a bimodal curve. Diagram 1 was made from such data except that the diagram does not include the data in table 18.

In diagram 1 there is a strong mode at 15 which corresponds to the new lethal, but the mode which corresponds to the original lethal gives only a weak mode at 22; indeed the curve is not obviously bimodal, small number of determinations not being sufficient to distinguish clearly between the two lethals. The

TABLE 18

NO. OF PAIR	FEMALES				MALES				LINKAGE OF WHITE WITH LETHAL	LINKAGE OF LETHAL WITH MINIATURE
	White- cos' n long	Eosin miniature	White- eosin miniature	Eosin long	Eosin miniature	White miniature	Eosin long	White long		
1	28	32	17	10	27	4	10	0	9.8	24.4
2	34	22	24	11	32	7	7	2	18.8	18.8
3	35	24	17	18	29	9	6	0	20.5	14.0
4	39	35	14	9	29	4	9	0	9.5	21.4
5	81	62	69	36	87	15	23	1	12.7	19.0
6	66	82	42	25	74	17	20	3	17.5	20.1
7	31	11	25	7	25	4	5	0	11.8	14.7
8	34	30	26	17	41	2	9	0	3.8	17.3
9	39	42	22	10	42	5	8	0	9.0	14.4
10	48	48	51	27	59	9	18	0	10.46	20.9
11	28	40	24	15	25	5	10	0	12.5	25.0
12	47	44	19	16	48	6	15	0	8.7	21.7
13	17	23	14	17	26	6	10	0	14.3	24.3
14	44	24	21	11	35	6	10	0	11.7	19.6
15	88	62	62	31	63	12	23	0	12.2	23.4
16	93	78	37	30	100	10	30	1	8.0	22.0
17	113	120	63	80	101	25	37	1	15.6	23.1
18	104	105	70	78	83	21	33	3	17.1	23.5
19	58	42	39	34	49	15	17	3	21.4	23.8
20	87	73	51	46	78	17	18	1	15.8	16.6
21	60	60	41	38	44	9	13	1	14.9	20.9
22	60	44	38	22	47	16	18	2	21.6	24.0
23	48	45	45	22	30	9	13	1	19.0	26.5
24	48	35	38	23	58	11	17	1	13.8	20.7
25	95	76	54	26	76	26	19	3	23.4	17.7
26	58	49	39	29	56	13	14	1	16.6	18.0
27	56	35	24	37	64	21	11	3	24.2	14.1
28	63	60	49	37	69	20	16	2	20.5	17.0
29	54	44	48	33	47	4	11	2	9.4	20.3
30	52	39	38	24	55	13	19	1	15.9	22.6
31	49	38	37	25	53	5	13	0	7.0	18.3
32	53	54	29	16	49	2	9	0	3.3	15.0
33	65	45	47	30	71	14	15	1	14.8	15.8
34	107	54	67	38	100	21	26	3	16.0	19.4
35	101	54	67	29	78	21	28	0	16.4	22.0
36	69	48	52	30	63	16	23	2	17.3	24.0
37	58	24	66	25	50	8	9	3	15.7	17.0
38	57	38	47	25	57	17	17	0	18.7	18.7
39	63	45	41	33	54	18	13	1	22.1	16.2
40	28	28	19	16	39	8	12	0	13.5	20.3
41	60	36	27	26	28	6	4	2	20.0	15.0
42	64	34	51	32	71	15	23	1	14.5	21.9
43	26	32	45	25	51	8	13	2	13.5	20.2
44	52	50	46	34	57	24	10	1	27.0	12.0
	2198	2174	1780	1201	2421	524	685	48	6633	8656



determinations of table 18 are all of the crossover value white new lethal, and are expected to give a unimodal curve with the mode at 15.

#### SUMMARY

Lethal factors were found in inbred stock only. Fresh wild stock gave no unusual sex ratios.

The first lethal ( $l_{sa}$ ) was found in stock caught in 1911 and has its locus at 23.7. One female of this stock gave an extraordinarily high sex ratio; viz., 83 females to 3 males. That this extraordinary sex ratio is due to the presence of two lethal factors, one in each X chromosome, is shown by the fact that all (not half) the daughters gave a 2 to 1 sex ratio.

The new lethal ( $l_{sb}$ ) that appeared in the female with extraordinary sex ratio crossed over, in one case examined, to the X chromosome that carried the factor for white. Its locus is at 16.7. Two other lethals were found in the 1910 stock. The first ( $l_{sc}$ ) has its locus at 65.2. The other ( $l_{sd}$ ) differs from all other lethals in that the lethal bearing males emerge from the pupa case, and die almost immediately on becoming adult flies.

# AN ATTEMPT AT A PHYSICO-CHEMICAL EXPLANATION OF CERTAIN GROUPS OF FLUCTUATING VARIATION

JACQUES LOEB AND MARY MITCHELL CHAMBERLAIN

## I

There is a general tendency to visualize the factors which determine the hereditary characters as specific chemical compounds. If we wish to carry this view (with which we sympathize) beyond the limit of a vague statement, we must either try to establish the nature of these compounds by the methods of the organic chemist, or we must use the methods of general or physical chemistry and try to find numerical relations by which we can identify the quantities of the reacting masses or the ratio in which they combine. Attempts in this direction have been made by the suggestion of Loeb<sup>1</sup> that phenomena of growth belong in the group of auto-catalytic processes, and by T. B. Robertson's<sup>2</sup> and Ostwald's investigations supporting and enlarging this idea; by A. R. Moore's<sup>3</sup> attempt to show that in hybrids the velocity of development of the dominant character is slower than in the pure dominant breed; and by Loeb and Ewald's<sup>4</sup> proof that all the embryos of *Fundulus* have practically the same rate of heart beat at the same temperature. Since our new experiments are a sequence of this last mentioned paper, we may briefly discuss its contents.

<sup>1</sup> J. Loeb. Ueber den chemischen Character des Befruchtungsvorgangs Roux's Vorträge und Ausätze, Leipzig, 1908. Biochem. Ztschr., 2, 34, 1906.

<sup>2</sup> T. B. Robertson. Roux's Archiv, 25, 581, 1908; 26, 108, 1908; 37, 497, 1913. Am. Jour. Physiol., 37, 1, 1915; Robertson and Wasteneys, Roux's Archiv, 37, 485, 1913; Wo. Ostwald. Ueber die zeitlichen Eigenschaften der Entwicklungs-vorgänge, Leipzig, 1908.

<sup>3</sup> A. R. Moore. Roux's Archiv, 34, 168, 1912.

<sup>4</sup> J. Loeb and W. F. Ewald. Biochem. Ztschr., 58, 177, 1913.

C. G. Rogers<sup>5</sup> has shown that the heart beat of the embryo of *Fundulus* has a temperature coefficient of the order of the magnitude of a chemical reaction, i.e., that it practically doubles for an increase of temperature of 10°C. Loeb and Ewald found that the rate of heart beat is practically the same in each individual embryo (of a certain age) for a given temperature, varying only in very narrow limits; so that the rate of the heart beat of any of these embryos could be utilized as a thermometer. The authors explained this fact on the basis of general chemistry as follows: given a sufficient quantity of substrate the velocity of the reaction is in proportion to the mass of enzyme. If we suppose that the rate of the heart beat is determined by the velocity of an enzyme reaction—which supposition agrees with the temperature coefficient—we must conclude that all hearts of *Fundulus* embryos must have the same mass of enzyme, since they all beat at the same rate when the temperature is the same. If we consider the rate of heart beat of the *Fundulus* embryo a hereditary character—which is legitimate—we are forced to the conclusion that each embryo of *Fundulus* inherits practically the same mass of those enzymes which are responsible for the heart beat. The hereditary factor in this case must consist of material which determines the formation of a given mass of these enzymes, since the factors in the chromosomes are too small to carry the whole mass of the enzymes existing in the embryo or adult.

## II

While the rate of heart beat is approximately the same in each egg (at the right age) and for the same temperature, we notice slight variations, the usual fluctuating variation. It occurred to us that this fluctuating variation might offer a chance for further testing the enzyme conception of the factors of certain hereditary characters. We selected, instead of the rate of heart beat, the velocity of cell division. Loeb<sup>6</sup> had shown in a former paper that the time from insemination to the first cell division

<sup>5</sup> C. G. Rogers. *Am. Jour. Physiol.*, 28, 81, 1911.

<sup>6</sup> J. Loeb. *Pflüger's Archiv*, 124, 411, 1908.

in the egg of the sea urchin *Strongylocentrotus purpuratus* can be so sharply measured and is so nearly constant that it can be used for the establishment of a temperature coefficient and this was later confirmed by Loeb and Wasteneys<sup>7</sup> for the egg of *Arbacia*. Since the influence of temperature is again of the high order characteristic of chemical reactions, we may make the assumption that each egg carries a definite mass of one or more enzymes or catalysts which determine the rate of cell division. If we fertilize a mass of eggs of the same female of *Arbacia* and keep them at the same temperature, we find that they do not all begin to segment at the same time, and that there is an interval between the cell division of the first and last egg of the group. If we assume that the velocity of the cell division is determined by the mass of enzymes and the temperature, the fact that at  $t^{\circ}$  some eggs divide after 100, others after 101, 102, until, e. g., 113 minutes, we must conclude that this difference in time is the expression of a corresponding difference in the mass of enzymes in different eggs, those dividing in 100 minutes having a greater mass of enzymes than those dividing in 102, 103, etc., and 113 minutes; and that the mass of enzymes varies in inverse proportion to the time required for cell division at a given temperature. On this basis we should have to assume that the latitude of variation in the rate of cell division of a group of eggs is the expression of a corresponding variation in the mass of enzyme in the individual eggs. This idea can be put to a test with the aid of the temperature coefficient. If we call  $m$  the minimum mass of the enzyme responsible for the first cell division in the slowest eggs, then we shall find a certain greater percentage of eggs with the enzyme mass  $m + a$ , a still larger percentage with the mass  $m + a_2$ , and a small number with the mass  $m + a_n$  where  $m + a_n$  is the greatest mass of enzyme occurring in an egg. If the eggs with the mass  $m + a_n$  divide at the temperature  $t^{\circ}$  after 100 minutes, they will divide in about  $Q_{10} \times 100$  minutes at the temperature  $(t - 10)^{\circ}$ , where  $Q_{10}$  is the temperature coefficient for  $10^{\circ}\text{C}$ . at this point; the eggs with the smallest mass of enzyme

<sup>7</sup> J. Loeb and H. Wasteneys. Biochem. Ztschr., 36, 345, 1911.

$m$ , which at  $t^\circ$  divide after 113' will divide at  $(t - 10)^\circ$  after  $Q_{10} \times 113$  minutes, since the temperature coefficient must be the same for both types of eggs. If we call the difference in the time of segmentation between the slowest and fastest egg the *latitude of variation*, this latitude of variation should vary in direct proportion to the temperature coefficient for cell division if our theory is correct.

### III

We will first give the temperature coefficient of cell division for the egg of *Arbacia* for different temperatures; i.e., the results of measurement of the time required from the moment of insemination to the moment when the first egg in the field was seen to divide. The eggs had been kept in a water bath with constant temperature, and a little before the cell division was expected to occur (which time we knew from the former observations of Loeb and Wasteneys) the eggs were put into a watch glass of the temperature of the eggs and the exact time ascertained when the first egg of the lot underwent cell division. Table 1 gives these times according to Loeb and Wasteneys, and according to our own observations. The reader will notice how closely both values agree.<sup>8</sup> Our values are the average of a number of determinations, which show only a negligible variation.

Beyond  $31^\circ$  no segmentation occurs. We tried no experiments on the latitude of variation beyond  $25^\circ$  or below  $9^\circ$ , since outside of these limits the segmentation is no longer entirely normal.

From the results of table 1 we compute the temperature coefficients for the time from insemination to the first appearance of cell division (table 2).

In order to determine the latitude of variation of the time of segmentation—i.e., the interval between the time at which the first egg of a set begins to segment and the time when the last egg segments for a certain temperature, we proceeded as

<sup>8</sup> The eggs were always used in the first hours after they had been removed from the animal. The time required for the first cell division was remarkably constant in different experiments. It is worth mentioning that such constancy is only possible when the temperature is kept constant.

TABLE 1

*Time in minutes from insemination to the cell division of the first egg in Arbacia*

TEMPERATURE <i>degrees</i>	LOEB AND WASTENEYS	LOEB AND CHAMBERLAIN
7.0	498.0	
8.0	410.0	411.0
9.0	308.0	297.5
10.0	217.0	208.5
11.0	175.0	175.0
12.0	147.0	148.0
13.0		129.0
14.0		116.0
15.0	100.0	100.0
16.0	85.5	
17.5	70.5	
18.0	68.0	68.0
19.0		65.0
20.0	56.0	56.0
21.0		53.3
22.0	47.0	46.0
23.0		45.5
24.0		42.0
25.0	40.0	39.5
26.0	33.5	
27.5	34.0	
30.0	33.0	
31.0	37.0	

follows: The eggs were inseminated in sea water, and kept in a water bath at the desired temperature. The eggs remained in this water bath until about the time when the first segmentation was expected to occur. In the meantime, a second water bath was prepared on the stage of the microscope whose temperature was slightly below that of the desired temperature. This water bath contained the watch glass in which the segmentation of the eggs was to be observed. The watch glass had therefore the temperature at which the eggs were observed. The temperature of this water bath was also kept constant. When the temperature at which the latitude of variation was observed was very low and that of the air of the room was high a slight error crept in, in as much as the temperature of the

TABLE 2

TEMPERATURE COEFFICIENT FOR	
8/18	$\frac{410}{68} = 6.0$
9/19	$\frac{297}{65} = 4.5$
10/20	$\frac{208.5}{56} = 3.7$
11/21	$\frac{175}{53.5} = 3.3$
12/22	$\frac{146}{46} = 3.2$
13/23	$\frac{129}{45.5} = 2.8$
14/24	$\frac{116}{42} = 2.8$
15/25	$\frac{100}{40} = 2.5$

water in the watch glass rose slightly during observation. This error made itself felt in that in the case of low temperatures the actual temperature was occasionally a trifle higher than intended. We shall come back to this point later on.

When the eggs had been put into the watch glass, a field with no less than 80 and often as many as 150 eggs was selected, and every minute the number of eggs which underwent cell division was counted until the last egg had divided. Very often a small percentage of the eggs had remained unfertilized and these of course did not divide.<sup>9</sup> In table 3 we give a few examples of the actual measurements of the latitude of variation in the time required from the segmentation of the first to that of the last egg in a field.

As far as the irregularities in the first two minutes are concerned, they must probably be attributed to the fact that the entrance of the spermatozoa into the eggs occurred somewhat irregularly, the moment of insemination differing in various eggs within one or two minutes. Table 4 gives the latitude of varia-

<sup>9</sup> When this number was great the material could not be used since in such cases the spermatozoa no longer entered the eggs simultaneously.

TABLE 3  
*Latitude of variation in segmentation time*

NUMBER OF EGGS SEGMENTED AFTER	TEMPERATURE					
	25°	15°	22°	22°	12°	12°
	Number of eggs in field					
minutes	117	127	116	126	116	100
1	3	1	1	2	4	3
2	12	6	8	24	15	5
3	34	15	21	49	26	8
4	68	34	33	85	40	10
5	107	44	85	95	51	12
6	10 eggs not fertilized	62	103	111	60	16
7		79	110	117	67	19
8		90	116	119	77	20
9		95			80	24
10		100			80	28
11		109			88	32
12	18 eggs not fertilized				88	36
13					88	38
14					90	49
15					92	60
16					95	75
17						84
18					100	85
19					101	
20					105	85
21					105	95
22					106	96
23					108	
24					8 eggs not fertilized	98
25						2 eggs not fertilized

tion, i.e., the difference in time between the segmentation of the last and that of the first egg in a field for different temperatures for all observations made. The averages appear in the last line.

This series illustrates the source of error to which we have already alluded, namely, that at low temperatures the times

TABLE 4

*Differences in minutes between segmentation of first and last egg in a field at*

9°	10°	11°	12°	13°	14°	15°	18°	19°	20°	21°	22°	23°	24°	25°
50	39	25	22	20	17	13	12	14	10	8	8	9	7	5
49	40	26	20	18	19	12	11	13	10		7	7	9	5
47		27	22	(13)	16	12	13	11	9		8	9	7	5
64				19	18	12		12			9	8	7 <sup>1</sup> <sub>2</sub>	
60				20		14		12			7	8	9	
46				18		14		14			8	7	8	
				20		12					8			
						14					8			
						14					7			
											8			
											8			
Mean 52.6	39.5	26	22.5	19.2	17.5	13	12	12.5	9.6	8	7.8	8	8	5 Min.

were liable to be too short when the outside temperature was very high. Thus the value 13 minutes for the temperature of 13° is unquestionably too low, and probably the values 46 and 47 for 9°C. are also too low. At the higher temperatures the values differ much less, since the temperatures approximate much more the room temperature.

We are now in a position to compare the expected with the observed result. The expected result is the series of temperature coefficients for the time from insemination to the time when the first egg of the set begins to divide; the observed result is the series of temperature coefficients for the latitude of variation, i.e., the time which elapses between the segmentation of the first and last egg in a set. These two sets of coefficients should be identical and table 5 shows the degree of agreement..

A comparison shows that the temperature coefficients for the latitude of variation are practically identical with the temperature coefficients for cell division, and that where a noticeable difference exists it is always in the same direction, namely, the coefficients for the latitude of variation are a trifle too small. We can account for this on the basis of the deficiency in the method we have already discussed, namely that when the temperature of observation was low and that of the room high, the

TABLE 5  
*Temperature coefficients for latitude of variation*

TEMPERATURES	EXPECTED	FOUND
9/19	4.7	$\frac{52.6}{12.6} = 4.2$
10/20	3.8	$\frac{39.5}{10} = 3.9$
11/21	3.3	$\frac{26}{8} = 3.2$
12/22	3.1	$\frac{22.5}{7.8} = 2.8$
13/23	2.8	$\frac{19.2}{8} = 2.4$
14/24	2.8	$\frac{17.5}{8} = 2.3$
15/25	2.5	$\frac{13}{5} = 2.6$

temperature in the watch glass may have risen slightly during the observations. Since in the determination of the temperature coefficient the value for the low temperature forms the numerator, it is obvious that the observed temperature coefficients are liable to be a little smaller than they would be without this error. We expect to test this idea next season.

#### THEORETICAL REMARKS

It was found in a previous investigation that the time which elapses from the moment of insemination to the moment of the beginning of cell division in the egg of *Arbacia*, is a constant for a given temperature. On the basis of the enzyme theory this was to be explained on the assumption that the mass of ferments contained in the egg of the sea urchin responsible for this process is approximately constant in each individual egg. This would mean that the hereditary factor determining the rate of cell division consists in determiners for definite quantities of ferments. This idea was put to a test by applying it to the fluctuating variability of this process. While for a given temperature the eggs of *Arbacia* will always begin to segment at the same time, not all the eggs segment simultaneously. Assuming

that those eggs which segment first have a greater mass of ferment than the others, fluctuating variability would in this case be due to differences in the mass of ferment in the different eggs of the same female. If this idea were correct, eggs with the maximum and with the minimum amount of ferment should differ in the rate of segmentation by an amount of time which would vary in direct proportion to the temperature coefficient for the process of segmentation. This theory was tested and it was found that the observed values agree very closely with the expected values: the slight variations found being in the direction of the possible source of error of the method of the experiments. These experiments support therefore the idea that the hereditary factor responsible for the rate of segmentation is a determiner for a given mass of certain ferments, and that fluctuating variability depends in this case upon slight but definite variations in the mass of those ferments in different eggs.

#### SUMMARY OF RESULTS

1. It is shown that the temperature coefficient for the latitude of variation of the segmentation of the egg of *Arbacia* (i.e., the time between the segmentation of the first and last egg of a group fertilized at the same time) is practically identical with the temperature coefficient for segmentation.
2. It is shown that the fact is intelligible on the assumption that the fluctuating variation in this case is due to a variation in the mass of enzyme contained in the different eggs and supposed to be responsible for the rate of segmentation.

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